OPEN ACCESS

University of BRISTOL

This electronic thesis or dissertation has been downloaded from Explore Bristol Research, http://research-information.bristol.ac.uk

Author:
Hedges, Virginia

Title:
Epidemiological and biomechanical studies into the role of biotin supplementation on lameness in dairy cows.

General rights
Access to the thesis is subject to the Creative Commons Attribution - NonCommercial-No Derivatives 4.0 International Public License. A copy of this may be found at https://creativecommons.org/licenses/by-nc-nd/4.0/legalcode This license sets out your rights and the restrictions that apply to your access to the thesis so it is important you read this before proceeding.

Take down policy
Some pages of this thesis may have been removed for copyright restrictions prior to having it been deposited in Explore Bristol Research. However, if you have discovered material within the thesis that you consider to be unlawful e.g. breaches of copyright (either yours or that of a third party) or any other law, including but not limited to those relating to patent, trademark, confidentiality, data protection, obscenity, defamation, libel, then please contact collections-metadata@bristol.ac.uk and include the following information in your message:

• Your contact details
• Bibliographic details for the item, including a URL
• An outline nature of the complaint

Your claim will be investigated and, where appropriate, the item in question will be removed from public view as soon as possible.

EPIDEMIOLOGICAL AND BIOMECHANICAL STUDIES INTO THE ROLE OF BIOTIN SUPPLEMENTATION ON LAMENESS IN DAIRY COWS

by

Virginia Hedges

A dissertation submitted to the University of Bristol in accordance with the requirements for the degree of Doctor of Philosophy in the Faculty of Medicine

Department of Clinical Veterinary Science

June 2001

words: 52,506

ABSTRACT

A longitudinal prospective intervention study investigated the effect of biotin supplementation on the incidence of lameness in a total of 900 milking cows and heifers on five commercial farms. Each farm participated for 18 months with a total of 1120 cow years of data. Within each herd the cows were randomly allocated to either receive a supplement of 20mg biotin per day or not and all cows were run as one herd on each farm. The overall incidence rate of lameness was 68.9 per 100 cows per year with a range of 31.6 to 111.5 by farm. The incidence rates of the four most frequently reported causes of lameness were sole ulcer 13.8, white line separation 12.7, digital dermatitis 12.0 and interdigital necrobacillosis 7.1 all per 100 cows per year. The key finding of the trial was that the risk of lameness caused by white line separation was approximately halved in cattle supplemented with biotin; Cox proportional hazard survival analysis hazard ratio = 0.59.

In a further study the white line was examined using biomechanical techniques. A total of 14 cows were randomly allocated to receive a supplement of 20mg biotin per day or not and again the cows were run as one herd. Samples were collected from zones 2 and 3 of the lateral and medial digits of both hind claws. The tensile strength of zone 2 (5.27 MPa sd 1.86) was significantly higher than that of zone 3 (3.11 MPa sd 1.80) ($P<0.01$) and the medial claw (4.75 MPa sd 2.07) had a significantly higher tensile strength than the lateral claw (3.64 MPa sd 2.03) ($P<0.01$). This complements observations of white line separation lameness which is typically seen at zone 3 on the lateral claw.

ACKNOWLEDGEMENTS

I would like to express gratitude to my supervisor Dr L E Green for all of the invaluable advice, support and help she provided throughout the study.

I would like to express my appreciation and thanks to Mr A J Packington, Mr R W Blowey, Dr W Steinberg and Dr G Weber for introducing this research project and for their continual support and advice throughout the study.

I would like to express my gratitude to Dr R Bonser for his advice and training in biomechanical testing techniques.

I gratefully acknowledge the farmers who have shown interest, cooperation and understanding, and this study would not have been possible without their collaboration.

I would like to express my thanks to the study veterinarians Mr R W Blowey, Mr G Stephens, Mr P Cunliffe and Mr C Watson, for their continual interest and collection of the valuable lameness data.

I would like to acknowledge Mr K Dunn and Mr G Dunn of Gee Dee Systems who designed the in-parlour system and continued to provide technical support.

I wish to express my gratitude to Dr C J O'Callaghan for valuable advice on statistical matters.

I would like to thank Mrs C Girdler for her constructive criticism and advice in the final stages of writing the report.

Finally I would like to thank my fiancé for his unconditional support, encouragement, constructive criticism and patience.

Financial support for the present study has been received from F Hoffmann-La Roche Ltd, Switzerland/Roche Vitamins Ltd, UK.

DEDICATION

This work is dedicated to the memory of my late father

William Gregory Hedges

DECLARATION

I declare that apart from the advice and assistance acknowledged, the work in this dissertation is my own and was carried out in accordance with the Regulations of the University of Bristol. The work is original except where indicated by special reference in the text and no part of the dissertation has been submitted for any other degree.

Any views expressed in the dissertation are those of the author and in no way represent those of the University of Bristol.

The dissertation has not been presented to any other University for examination either in the United Kingdom or overseas.

SIGNED: ..... DATE: ..30/10/01.....

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance	Suppl	Biotin supplemented
ATP	Adenosine triphosphate	Unsuppl	No biotin
CI	Confidence interval	N	Number
CoA	Coenzyme A	Q1	1st quarter
DAISY	Dairy information systems	Q3	3rd quarter
DHIA	Dairy Herd Improvement Association	SD, sd or St Dev	Standard deviation
DNA	Deoxyribonucleic acid		
FAWC	Farm Animal Welfare Council		
GMP	Guanosine monophosphate		
ICS	Intercellular cementing substance		
ID	Identification		
MCG	Membrane coating granules		
MCM	Membrane coating material		
mRNA	Messenger ribonucleic acid		
NMR	National milk records		
P	Significance probability		
PCA	Plate count agar		
RBCA	Rose bengal choloramphenicol agar		
RNA	Ribonucleic acid		
SCC	Somatic cell count		
SE	Standard error		
TB	Tuberculosis		
TCA	Tricarboxylic acid		
UK	United Kingdom		
VHN	Vickers hardness number		
VRBA	Violet red bile agar		
WL	White line lesion		
%	Percentage or proportion		

CHAPTER ONE

GENERAL INTRODUCTION

Lameness in dairy cattle is still of considerable welfare and economic concern to farmers in the UK. It is widely believed to be attributable to high production and the associated intensive management and nutrition (Vermunt 1990; Greenough and Weaver 1997; Weaver 1997). A recent report by the Farm Animal Welfare Council (FAWC) (December 1997) justified the need for further investigations into lameness to reduce the severe economic effect on farm production and improve the welfare of the cow.

Lameness incidence to date

Surveys and studies have identified what appears to be an increasing lameness problem over time. In 1977 a national survey carried out in the UK found a lameness incidence of 5.5% (Russell *et al* 1982). The incidence in the 1980's was estimated to be 25% per year according to Greenough and Weaver (1997). Distl (1995) concluded that more than 50% of all cows suffer from foot and leg problems in European countries. Following a study on 37 farms in England and Wales, Clarkson *et al* (1996) found that 60% of dairy cows were lame each year, incidence rates of 55 new cases per 100 cows per year and a prevalence rate of 20%. However DAISY, which reports collected information from participating veterinary practices, reported 36% lameness in 63 dairy herds from a report published in 1993. More recently DAISY data from 50 Holstein and Friesian herds in southern UK found on average 25.6% lame cows in a typical 100 cow herd, with no

herd size effect (Kossaibati and Esslemont 2000). However, epidemiological techniques have been utilised to identify the determinants of lameness but there is a need for large-scale epidemiological studies of lameness. Studies to date use a variety of data, or variable gathering and processing methods, which are not easy to compare. It is evident that lameness continues to be a major problem on farms in the UK.

Epidemiology and study design

Studies to date on lameness incidence or prevalence have used varying statistical techniques, univariate and multivariate such as logistic regression. However, more recent advances in epidemiological and statistical techniques have provided the tools for more complex and complete analysis of lameness data. Studies may be enhanced by the ability to design more complex studies, appropriate calculation of sample sizes or number of events of interest (e.g. sample size and length of follow-up), (Altman 1991) the use of well defined outcome variables and more advanced analytical techniques such as survival analysis, for example Cox and Weibull models, which have been recently applied to the data of Clarkson *et al* (1996) by Hirst *et al* (2000).

As lameness is multifactoral and time is an important factor, for example, seasons, calving and stage of lactation, survival analysis is a very valuable tool. The advantage of survival analysis, a relatively new technique to lameness data, such as Cox proportional hazard survival analysis (semiparametric regression model) (Cox 1972), is the ability to consider the data in a time to event format, with the relative risks of independent variables built in a multiplicative fashion on the baseline (thus proportional) (Altman 1991; Hosmer and Lemeshow 1999). So significant associations and interactions can be

identified. Additionally a Kaplan-Meier survival curve plots the survival data in a stepwise format and displays the probability of surviving a given length on time, which complements the Cox model (Altman 1991). It is particularly useful in intervention studies for identifying differences between groups. Survival analysis methods can investigate risk much more thoroughly than for example logistic regression (limited to a positive or negative outcome) (Hirst *et al* 2000), when time to event is known.

French (2000) identified that many studies have also restricted risk factors in intervention studies to one or two related to the disease. It is important that in all observational and intervention studies all confounding factors are considered in any analysis, because a false conclusion may be found otherwise, and a well designed intervention study can reduce the confounders by design, for example, within herd analysis and measurements such as lactation number, known to be associated with lameness, are recorded and tested .

As Clarkson *et al* (1996) pointed out, there are many pitfalls that occur in various methods of data collection with particular reference to lameness. This includes data capture by veterinary surgeons only, versus studies involving both veterinarians' and farmers' observations which may result in incomplete data. As Weaver (1998) pointed out from the results of a postal questionnaire, a great proportion of lameness cases are treated solely by the farmer himself. Specifically trained observers have been reported to present 80% accuracy in locomotion scoring, which is reported to be more preferable to, than reducing the sample population so that it is manageable for one researcher (Murray *et al* 1994). Some programmes have been devised for recording causes of lameness accurately (French 2000) although some have been biased toward selecting farms with a

relatively high standard of management for the programme (Greenough and Weaver 1997).

A good epidemiological study also relies on a sound knowledge of the previous research of the subject of interest.

The economic costs of lameness

Substantial financial losses can result from lameness experienced in a herd, third only to poor fertility and mastitis (Kossaibati and Esslemont 1997). It has been estimated recently by Kossaibati *et al* (1999) that a single case of sole ulcer costs approximately £246 and other horn diseases approximately £151. A case of skin disease in the foot was estimated at approximately £58. The total average being approximately £136 per limb case and approximately £152 per affected cow. The actual costs for an incidence of lameness can be crudely divided into direct losses from a drop in the milk yield of the cow, losses of milk due to treatment, veterinary visits and treatment and the herdsman's time; indirect costs include culling, extended calving interval and problems with conception and therefore increased services (Kossaibati and Esslemont 1997). Lame cows are estimated to suffer 1.5 cases of lameness per year on average (Kossaibati and Esslemont 1997, 2000). Therefore it has been estimated that an average loss of £4000 per 100 cows per year can be encountered (Kossaibati *et al* 1999).

The bovine claw

Understanding of the structure and function of the 'normal' healthy claw is important when considering the claw disease processes and prevention.

Christine Warzecha (1993) investigated the structure of the ruminant hoof which was the main reference for the following section and further detail may be found within her thesis. The main components of the claw can be crudely divided into six categories by design:

- The coronet; interdigital space; wall; heel; sole; internal aspects.

Structures of the claw are shown in Figures 1.1 and 1.2.

Claw horn grows 0.5 cm a month on average, new coronary horn takes approximately 15 months to reach the weight bearing surface (Prentice and Neal, 1972; Tranter and Morris, 1992; Blowey, 1993; Schmid and Geyer 1994; Blowey and Greenough, 1998). Sole horn takes approximately 3 to 4 months to reach the ground surface from dermis and production depends largely on the depth of the sole (Schmid and Geyer 1994). All horn growth may vary however (0.3-0.7 cm per month) according to environmental temperature, age of the cow and nutrition. Differences in hind claw to fore claw and medial and lateral claw growth and wear rates have been suggested but are still inconclusive (Vermunt and Greenough 1995b).

The apical heel horn, commonly considered or termed sole material, is approximately 0.3-1 cm thick and the soft, flexible heel bulb approximately 0.5-1.5 cm thick. Growth rates in this region are difficult to estimate because horn is produced (palmar and plantar) and pushed over the deeper layers produced apically. The heel bulb structure has been reported by some workers to differ in different breeds of dairy cow,

therefore it has not been recommended as a monitoring material for claw quality (Warzecha 1993 cited Schroder 1970 and Fuchs 1976). It is known that the renewal time of this region is shorter than the renewal of the wall horn (Warzecha 1993). Schmid and Geyer (1994) found heel growth of 5mm per month.

The coronet

The coronet or perioplic epidermis layer is 1.5 cm (depth) of soft dermis located at the skin-horn junction. This consists of tubular and intertubular horn with loosely packed cells. This is produced from the coronary segment where the stratum germinativum consists of the stratum basale and stratum spinosum below a well developed stratum granulosum (Warzecha 1993). It is permeable to water and water soluble components. Its main function is to protect the underlying vulnerable tubular horn. It commonly degenerates with increasing age in the cow. It can also become softer and potentially wear away with extreme weather or environmental conditions. These factors combined with trauma, may result in hoof lesions e.g. vertical wall fissures (Blowey 1993; Blowey and Greenough 1998).

Interdigital space

This is the area of attachment of the medial and lateral claws of the cow. It consists of cornified skin with a hair border on the dorsal, palmar and plantar border. The apical 2/3 of this region descends into the periople followed by the axial claw and into the heel on the palmar and plantar borders. Many lesions including for example, interdigital dermatitis, interdigital hyperplasia, fibromas and interdigital necrobacillosis can be seen

here. In the stratum corneum of this area there are microscopic fissures, small hollow spaces and cellular desquamation, especially near the surface (Warzecha 1993). This may allow ascending infection.

The wall

Or parietal epidermis can be divided into three sections:

- Stratum externum
- Stratum medium
- Stratum internum

The proximal edge of the wall consists of the coronary border which joins the hoof wall to the skin and hair of the limb and the most distal part of the hoof wall is the 'weight bearing' border which supports a large part of the cow's body weight (Warzecha 1993).

Stratum externum - is the perioplic horn layer, previously discussed. It extends approximately 1-2.5 cm wide around the proximal edge of the hoof (Warzecha 1993) (Figure 1.1 and 1.2).

Stratum medium - or coronary horn consists predominantly of tubular horn which generate from the papillae of the corium and move down to the bearing surface, and intertubular horn is produced between the papillae (Figure 1 and 2). This is the largest and strongest part of the horn (Warzecha 1993). The number of tubules averages 80 per mm^2 (approximately 75 per mm^2 outer zone, 80 per mm^2 middle zone and 35 per mm^2 inner zone) these are individually determined at birth and vary according to breed and the individual (Warzecha 1993).

These horn cells start from the stratum germinativum which are present on the papillae. They mature in the cytoplasm from the stratum basale to stratum spinosum where tonofibrils (commonly referred to as onychogenic substance) radiating from the desmosomes (intercellular adhesions) can be observed. The crossing of the tonofibrils form an internal cytoskeleton which aids both strength and flexibility. The matured, hardened layer is known as the stratum corneum, which constitutes the major part of this section (Warzecha 1993; Blowey 1993; Blowey and Greenough 1998).

The tubules consist of completely differentiated horn cells containing keratin which flatten to surround the marrow centre as shown in Figure 1.3 (Warzecha 1993). There is only one type of tubule in the cow (Type I) unlike the horse (Bolliger 1991).

The intertubular horn produced from the stratum germinativum again consists of cells containing keratin which are degenerated epidermal cell components. Its structure is much softer and liable to weakening. The absolute structure of these tubules and intertubular substance can differ greatly with their position within the hoof (Figure 1.3) (Warzecha 1993).

Stratum internum - The most internal aspect of the hoof wall is the lamellar epidermis or stratum internum, a non-branching or primary leaflet area found in the distal half of the wall segment (Warzecha 1993). It consists of corial leaflets and epidermal or horn leaflets which interlock in an s shape, and produce a wave-like formation around the claw. These leaflets run approximately at right angles to the exterior horn wall except for the axial leaflets which are arranged apically (Warzecha 1993).

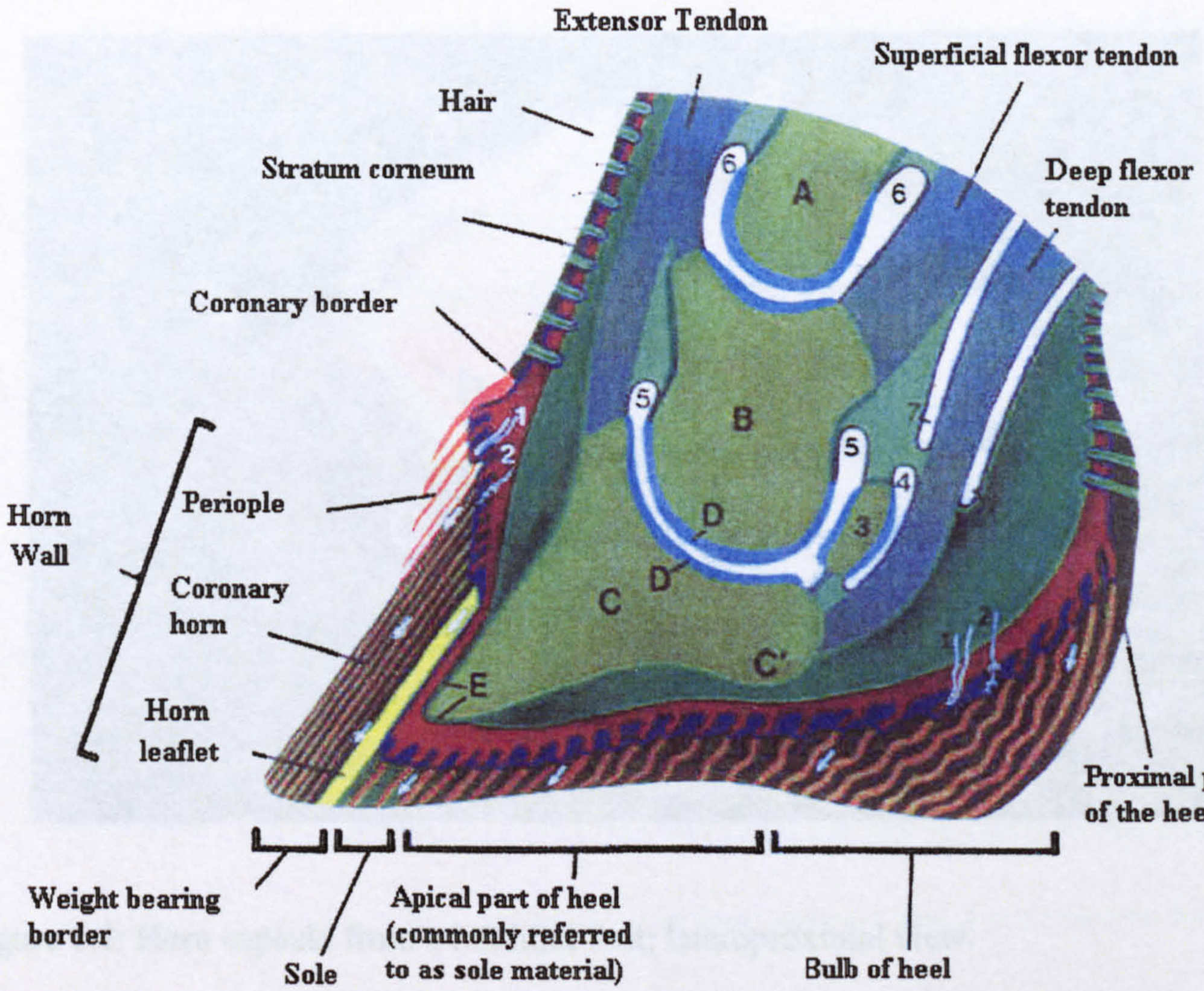


Figure 1.1: Longitudinal section of the cows claw

A, First phalanx
B, Second phalanx
C, Third phalanx
C', Tuberositas flexoria
D, Cartilage
E, Periosteum

1, Blood vessels
2, Nerves
3, Sesamoid
4, Bursae
5, Joint capasule of distal joint
6, Joint capsule of middle joint
7, Tendon sheath

Subcutis

Stratum germinativum (soft part of the epidermis)

Corium

Horn leaflets of the wall segment distally forming part of the white line

Direction of growth in the horn

(taken from Warzecha 1993)

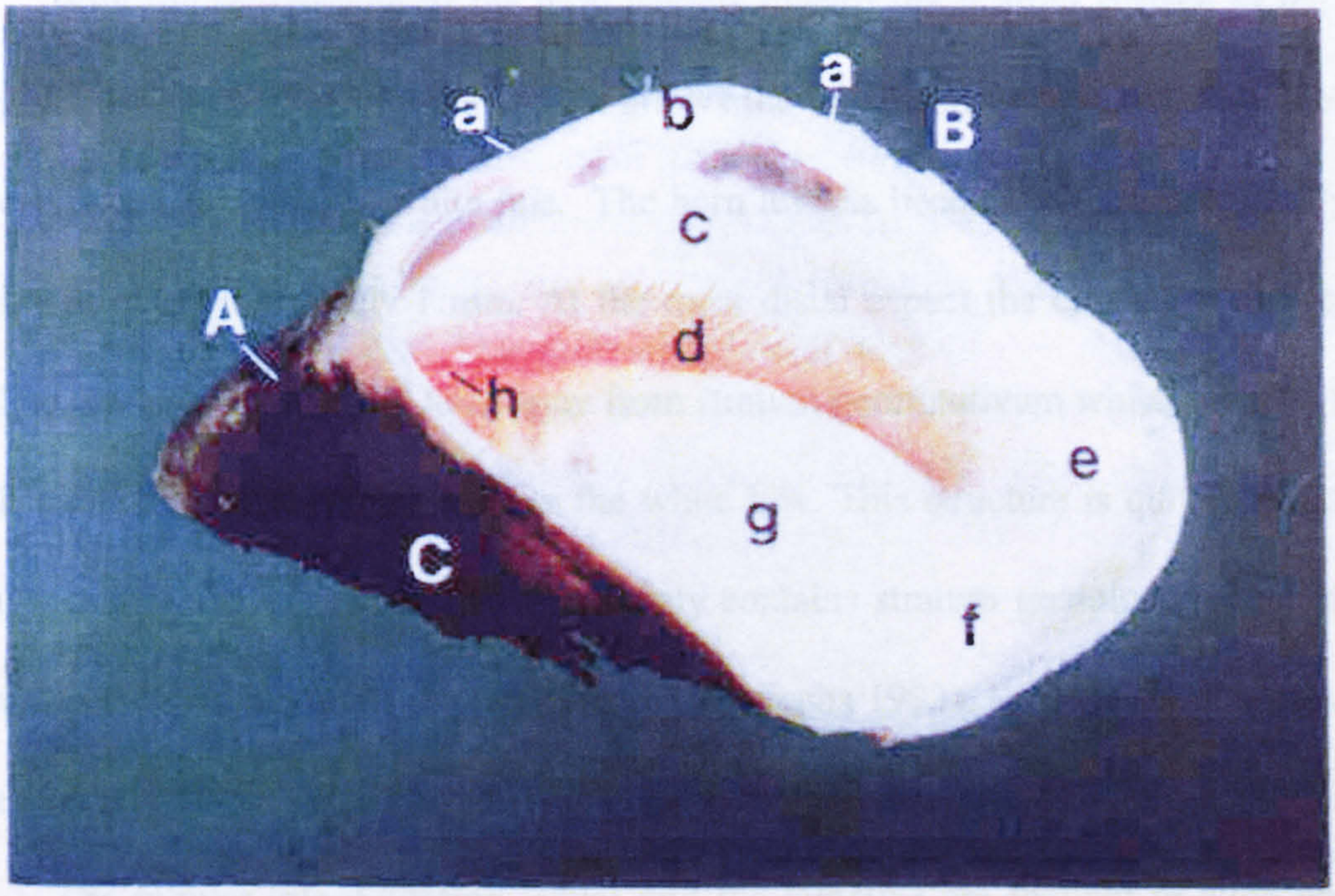


Figure 1.2: Horn capsule from a left hind foot; lateroproximal view.

a, Coronary border; b, Perioplic horn; c, Coronary horn; d, Horn leaflets; e, Transition from the perioplic to the coronary and heel segment; f, Bulb of the heel; g, Apical heel or sole; h, Sole horn; A, Dorsal part of the horn wall; B, Abaxial side wall; C, Axial side wall. (taken from Warzecha 1993)

The corial leaflets are covered in a layer of stratum germinativum (soft leaflets) and are approximately 100-150 μm wide, as opposed to the horn leaflets which are 40 μm wide and lie between the soft leaflets and are produced by the stratum germinativum. The horn leaflets then grow distal to the weight bearing surface along the soft leaflets (Dirks, 1985 cited by Warzecha 1993). This structure may be the most important attachment in locomotion as it fundamentally joins the claw capsule to the inner claw structure.

With the progression of the horn leaflets toward the bearing surface of the claw the corial leaflets become shorter, which allows the formation of cap horn from the horn leaflets associated with the white line. The horn leaflets become taller more distally to a maximum of approximately 1 mm. At the most distal aspect the corial leaflets become papillae producing a terminal horn layer from stratum germinativum which attaches to the cap horn as tubular horn, resulting in the white line. This structure is quite weak and is discussed later. This structure also commonly contains stratum granulosum between the stratum germinativum and stratum corneum (Warzecha 1993). Lamellar horn is produced quite slowly.

The tubular interdigitating leaves and the lamellae are thought to slide past the stationary cells of the keratogenic layers to give load support and movement in the hoof. The desmosomes that join the stationary to the moving parts break and reform in a staggered format. (Maclean 1971; Blowey 1993; Blowey and Greenough 1998; Ossent et al 2000).

The heel

The heel dominates the palmar and plantar aspect of the hoof and runs abaxially in a narrow band, but axially forming a third of the plantar or palmar aspect, and proximally to meet the periople. The apical part of the heel is fairly flat, quite hard and commonly referred to as part of the sole (Warzecha 1993).

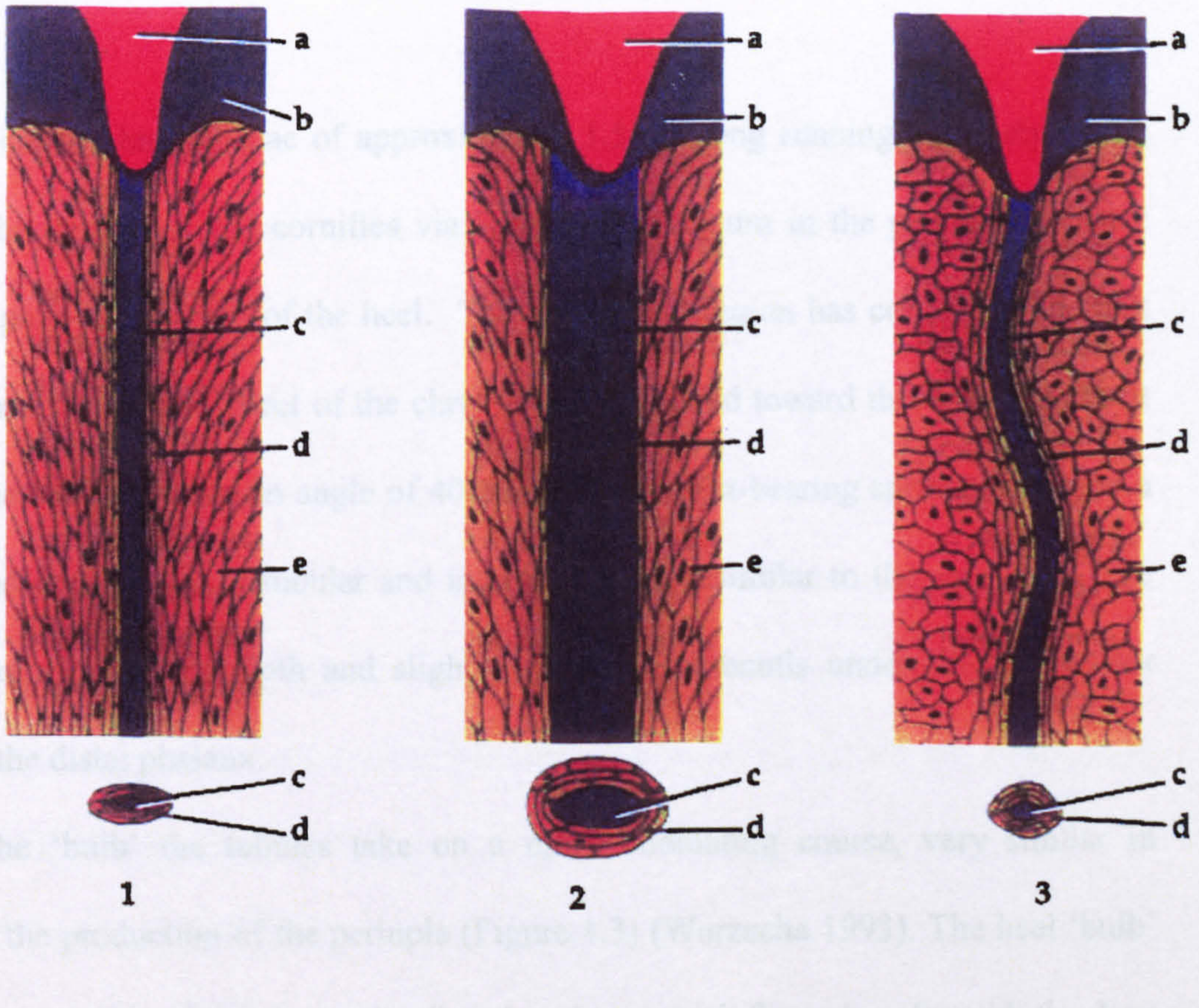


Figure 1.3: Longitudinal section and cross sections of tubules in different parts of the claw

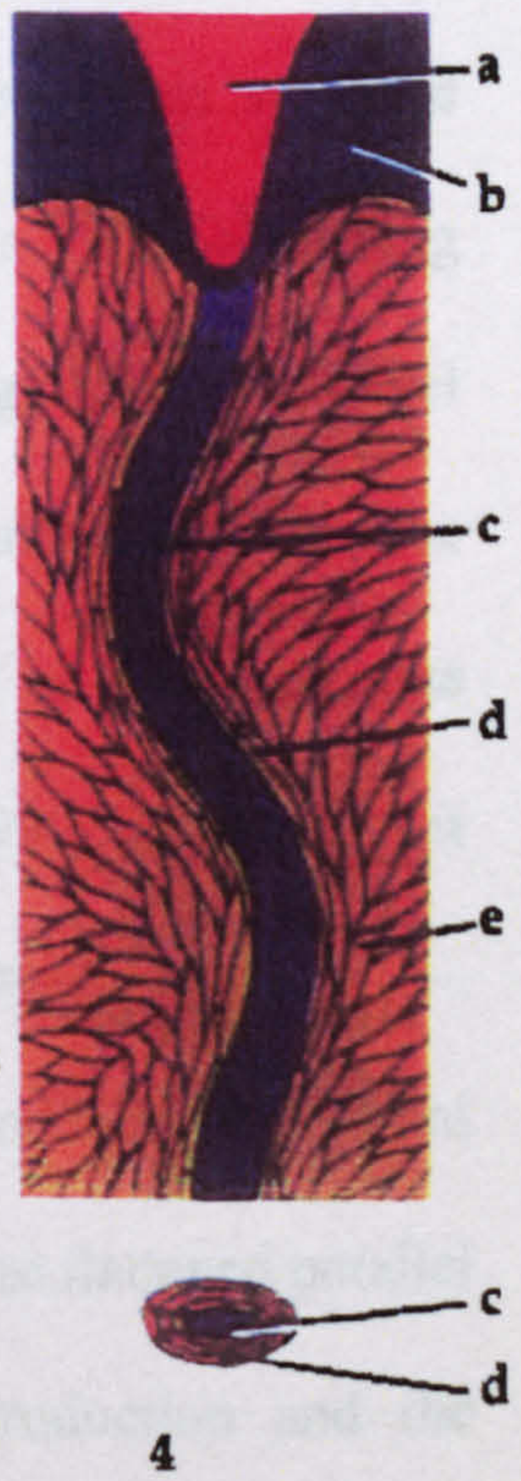
1 Horn tubule in the outer part of the coronary horn

2 Horn tubule in the middle coronary horn segment

3 Horn tubule in the inner coronary segment

4 Horn tubule in the bulb of the heel

a Corium
b Stratum germinativum
c-e Stratum corneum
c Marrow
d Cortex
e Intertubular horn



(taken from Warzecha 1993)

The corium has papillae of approximately 1.5mm long running obliquely to the surface of the epidermis and cornifies via stratum granulosum in the proximal, plantar and palmar parts of the bulb of the heel. The apical heel region has corial papillae that incline toward the dorsal aspect of the claw 60-70^0 and bend toward the horn tubules at their tip. The tubules run at an angle of 40-50^0 to the weight-bearing surface and have a structure and consistency of tubular and intertubular horn similar to the sole horn. The structure has a moderate depth and slightly developed subcutis under the *tuberositas flexoria* on the distal phalanx.

In the 'bulb' the tubules take on a more undulating course, very similar in structure to the production of the periople (Figure 1.3) (Warzecha 1993). The heel 'bulb' has a strong subcutis and envelopes the digital cushion which fuses dorsally with the deep digital tendon and axially with the fibres from the cruciate ligament. This structure inhibits drainage of the retroarticular space. The bulb region has a shock-absorbing function with lipid content, creating a permeability barrier, of 29.2g/kg, the highest level in foot material (Scaife *et al* 2000). It can expand abaxially or axially and forms the largest part of the weight-bearing surface, able to dissipate weight to the wall areas (Baggott and Russell 1981; Johnston 1990; Vermunt and Greenough 1990; Distl and Mair 1990; Blowey 1993; Greenough and Weaver 1997; Blowey and Greenough 1998).

In all regions of the heel the structure becomes less organised and cohesive toward the surface. Spaces and cracks are commonly observed. The tubules are flattened parallel to the surface and show signs of degeneration very early in production and the intertubular horn seldom contain nuclei (Warzecha 1993).

The sole

This structure consists of tubules which run down toward the weight bearing surface approximately 16-20 per mm^2 and an intertubular matrix (Blowey and Greenough 1998).

On average the sole is seven millimetres thick, although it is thicker at the junction with the bulb than it is at the apex where it joins the white line (Figure 1.1 and 1.2) (Blowey 1993; Blowey and Greenough 1998). The sole does not have a subcutis (Warzecha 1993). The corial region consists of 1 mm papillae that are arranged in rows with 1 mm stratum germinativum located between. The epithelial cells in the intertubular horn have a striped appearance as they are arranged in leaflets (Warzecha 1993).

There are often areas of degeneration viewed on the surface of the horn. These are commonly associated with the tubular structure, deteriorated cells and enlarged cortical structures (Warzecha 1993). The lipid content of this region is 23.9 g/kg (Scaife *et al* 2000).

White line (zona alba)

The white line is known mainly as the attachment of the sole to the wall on the ground surface (Figure 1.2). It runs from the heel bulb around the claw to the toe and for the first third of the axial wall (Blowey 1993; Blowey and Greenough 1998). The structure is described in detail in Chapter five.

There has been a concept called the 'sterile bed' which is widely referred to in cattle and horses although it has been disputed more recently by Budras *et al* (1989) in horses and (Budras *et al* 1996) for cattle. This concept states that the laminar portion of

the hoof is sterile and has limited or no ability to produce horn Budras *et al* (1996). These authors suggested that a small amount of activity occurs in the laminar portion in connection with their findings of increased horn production from the apex of the zona alba to its palmar or plantar end.

Internal aspects of the claw

Subcutis - The subcutis is located in areas of movement where 'padding' is required for the supporting structures. This material is not located in either the sole or wall region. Fat pads have recently been identified as a padding material in the sole (Lischer and Ossent 2000). The subcutis is well developed in the bulb of the heel, 5-10mm thick. It is dramatically thinner in the apical part of the heel. It is also found in the perioplic segment where it forms a ridge towards the coronary segment and it is pronounced at the dorsal and abaxial aspect and progressively thinner in the distal or palmar or plantar direction. The axial side of the subcutis is also thin (Warzecha 1993).

Corium. - Composed of stratum reticulare and stratum papillare, the corium is well vasculated and innervated and commonly termed the 'living' part of the claw. It serves nourishment to the avascular epidermis by diffusion from a system of papillae in the Stratum papillare and primary leaflets which provide a large surface area and bonding between the two sites (Warzecha 1993).

The coronary segment forms a band around the internal aspect of the hoof originating below the perioplic segment and extending down the hoof approximately 3cm dorsally to 1.5cm laterally (Bolliger, 1991). The papillae merge at their base to join the wall of the hoof in the horn leaflet area.

Membrane-coating material

Membrane coating granules (MCG) are synthesized in the deep layers of the stratum spinosum (Mulling *et al*, 1992 cited by Warzecha 1993). These have a lysosomal function and contain hydrolases (Hayward, 1976; Budras and Bragulla 1990 cited by Warzecha 1993). They are released via exocytosis into the intercellular space from the transition to the stratum corneum (Warzecha 1993). The membrane coating material (MCM) which represents the intercellular components, consists of various fractions of glycogen, glycoproteins and phospholipids and the carbohydrate fraction which aids in mechanical binding, (Budras and Bragulla 1990 cited by Warzecha 1993) and overall acts as an adhesive and deformable glue and represents the component responsible for horn quality (Warzecha, 1993; Budras *et al* 1998a). The lipid fraction, or phospholipids (Geyer 1984) are responsible for the permeability of the horn and therefore moisture equilibrium which may drastically change the biomechanical properties of the horn (Bertram and Gosline 1987; Scaife *et al* 2000).

MCM are infrequently observed in the outer layers of the coronary horn until the inner layer of the middle segment and the inner segment which contains small spaces containing this material (Warzecha 1993). The heel contains pools of MCM and narrow intercellular spaces. These spaces tend to enlarge slightly as they progress from the deeper tissues to the surface (Warzecha 1993).

Differences in fatty acid profile have been observed in claws of lame cows, possibly related to poor horn quality, or changes in the quantity and content of the intercellular cementing substance and therefore cell to cell adhesion, although this

particular study was in the preliminary stages (Offer and Logue 1998). Excessive formation of vesicles containing MCM also occurs in laminitis, which results in marked widening of the intercellular spaces (Budras and Bragulla 1990 cited by Warzecha 1993). Synthesis of poor quality MCM may occur with excessive cornification or abnormal production.

Unsurprisingly, therefore, many report that horn quality is inversely proportional to the width of the intercellular spaces and the amount of intercellular glue (MCM) (Budras and Bragulla 1990 cited by Warzecha 1993; Budras *et al* 1998a).

Keratinisation of claw horn

Keratins are present in all epithelial cells (keratinocytes). Post-mitotic cells of the stratum corneum are produced from the stratum basale, composed of mitotic cells, and formed in the coronary segment over the stratum spinosum; in the heel region stratum granulosum are also involved. During the process of keratinisation the cell organelles break down via hydrolysis. The resultant cytoplasm of the horn cells are then composed mainly of keratin fibrils and amorphous keratin proteins (Junqueira and Carneiro 1986 cited by Warzecha, 1993).

Keratin subtyping aids in the individual characterisation of cells and different sites of the claw are characterised by keratin protein patterns that have the final control over the moisture uptake in each area and therefore dictate the mechanical parameters (Bertram and Gosline 1987). The molecular weight of the different types helps to determine the hardness of the material (Bonser 1996). This has been seen more clearly in

tests carried out on equine hoof material, which has shown a differentiation between basal and suprabasal strata layer keratins (Hendry *et al* 1997).

Studies have identified the presence of 6 cytokeratins in bovine claw tissues, and the identification of low molecular mass acidic keratins a1-4 have also been located in differentiating epidermal cells and uncharacterised basic keratin, designated b2, although the precise location and structural role of many of them is still uncertain (Hendry *et al* 1997).

More details on keratins and their structure and function can be found in Chapter five.

Hoof pigmentation

Pigment in the horn cells originates from cells in the stratum basale and small accumulations of pigment granules are seen in the cells of the stratum spinosum. An even distribution of granules exists throughout the entire cells of pigmented horn and heavily pigmented claws, even the periople and interdigital space, may contain a small amount of pigmentation, although not in cap or terminal horn (white line) (Warzecha 1993).

Some workers have identified a difference with pigmentation and non-pigmentation and potentially reduced lesion severity (Baggott *et al* 1988; Chesterton *et al* 1989; Vermunt and Greenough 1995b), although other workers have disputed this claim (Clark and Rakes 1982; Douglas *et al* 1996).

Claw hardness in relation to pigmentation has been studied and discussed in Chapter five.

Lameness-causing lesions

70-90% of diagnosed causes of lameness are reported to be claw disorders (Lischer 1993 cited by Warzecha 1993), Kossaibati and Esslemont (2000) reported 60% of total lesions in the horn causing lameness and 36% affected the skin around the claw (data from DAISY). A study of 227 cows in one herd reported 86% hoof problems out of a reported 36% limb lameness in the herd in their productive life (Stanek and Stur 1984 cited by Warzecha 1993). Parizi and Hosseini (1998) similarly found 89 % of lameness was located in the claws in Shiraz, Iran, 73% hind limb, predominantly lateral claw and 27% fore limbs, predominantly medial claw.

Laminitis or coriosis

The precise aetiology and pathogenesis are still incomplete (Vermunt 2000) and more recently coriosis has been adopted as terminology more correctly descriptive of the pathogenesis than laminitis. The risks for laminitis include systemic disease, age, parity, genetics, conformation, stage of lactation, nutrition, housing and other management and environmental factors (Offer, McNulty and Logue 2000). These are interdependent factors which may have a direct or indirect effect individually or may combine to cause a problem, therefore the day to day life of the cow contains potential risk factors (Vermunt 2000).

The most recent pathological studies have been carried out by Ossent *et al* (2000) who identified that laminitis starts as a result of inflammatory mechanisms. This may result from a disturbance in the circulation in the bovine claw or corium from vasoactive substances, such as histamine and endotoxin or lowered pH, trauma or compressive

stress, potentially resulting in blood being shunted away from capillary circulation through dilated arteriovenous anastomoses. Due to vaso-paralysis the blood pools and stagnates and hypoxia causes leaking blood vessels which leads to oedema and haemorrhage (Ossent *et al* 2000, Vermunt 2000).

In addition to this an elongation, bending with contusion and mechanical restriction of the capillaries, can result from unsuitable weight loading on the claw. Hirschberg (2000) found that horn production sites are supplied solely by the dermal vessels. These are sensitive to minor disturbance in microcirculation leading to reduced diffusion of nutrients and oxygen and changes in the foot thickness and shape, which in turn leads to further microcirculatory disturbances. Therefore, haemorrhages observed commonly in heifers should be avoided to promote their long-term welfare and productivity, as cows with clinical laminitis often have a poor prognosis and shorter productive life (Bergsten 1994). First lactation lameness is indicative of increased lameness risk in future lactations (Bergsten 1994; Logue *et al* 1998c; Kerr 1998; Hirst *et al* 2000).

Oedema and haemorrhage may result in necrosis and lamellar junction disruption and weakening. Many believed that this alone leads to a drop in the distal phalanx within the claw, because natural locomotion forces it down. Ossent *et al* (2000) however, proposed that the forces required for the distal phalanx to drop exceeded those given by even the heaviest of animals. They suggested that hormones around pregnancy and calving relaxed or increased the elasticity of the collagen fibres of the suspensory apparatus or the structure that aids in the support of the lower limb, which in turn assisted the lowering of the distal phalanx.

When sinking the distal phalanx depresses the corium of the sole and heel and creates further capillary damage, haemorrhage, cellular inflammation and necrosis. Chronic oedema combined with the body weight of the cow present the same amount of potential damage as actual sinkage of the distal phalanx. However, a lesion only appears in the horn as the horn grows down the claw (Ossent *et al* 2000).

Inferior claw horn may occur as a result of chronic diffuse disturbances and in the claw of the older cow which has had common laminitic bouts a 'slipper foot' or concave furrowed dorsal wall claw may be observed (Ossent *et al* 2000).

Laminitis is more commonly encountered in heifers than older cows and the initial insult is observed to be around calving (Offer, McNulty and Logue 2000). This is proposed to be because heifers receive little or no concentrate until they are due to calve. They may also be introduced suddenly into a milking herd and hard abrasive surfaces, which are both risk factors for laminitis (Vermunt 2000). However, Vermunt and Greenough (1996a) found that the majority of Canadian heifers already had sole haemorrhage at 13 months of age and laminitis at different levels can be seen in the early growing stages (Vermunt and Greenough 1995a).

<u>Sole ulcer and heel ulcer</u>

The sole ulcer or Pododermatitis circumscripta is also known as the Rusterholz ulcer as it was first described by A. Rusterholz in 1920. This lesion is considered to be the most common and often recurs in lactations following the initial insult (Enevoldson *et al* 1991b; Clarkson *et al*, 1996, Lischer and Ossent 2000). It occurs more frequently in older

cows (Baggott and Russell 1981) and can be observed worldwide (Baggott and Russell 1981; Greenough and Weaver 1997).

Russell *et al* (1982) identified 12% of the total lesions per year were sole ulcers in the 1970's. Enevoldson *et al* (1991b) found 20% and 29.7% of cows had sole ulcer in one foot, or more than one foot respectively from a total of 2204 first lactation cows and 23.5% and 24.7% respectively in 1124 second to ninth lactation cows. Sole ulcer was responsible for 38% of lameness according to Kossaibati and Esslemont (2000). Murray *et al* (1996) however, described a 46% incidence of sole ulcer, significantly higher than the previous studies.

Sole ulcers are said to occur in approximately 50% of lactations in Friesian herds (Enevoldson *et al* 1991b), the majority of cases occurring between days 91 and 180 post-calving (Kossaibati and Esslemont 2000; Logue *et al* 2000). Logue *et al* (2000) also identified February and June as the most predominant months for sole ulcer occurrence (Baggott and Russell 1981).

The location of the sole ulcer within the cow's claw is very specific. This lesion appears at the junction of the sole or apical heel and the heel bulb on the sole surface of the claw. It is also located closer to the axial than the abaxial side of the claw because the laminar junction on the axial side is weaker than the abaxial side and therefore failure is proposed to rotate the third phalanx within the claw (Greenough and Weaver 1997; Blowey *et al* 2000).

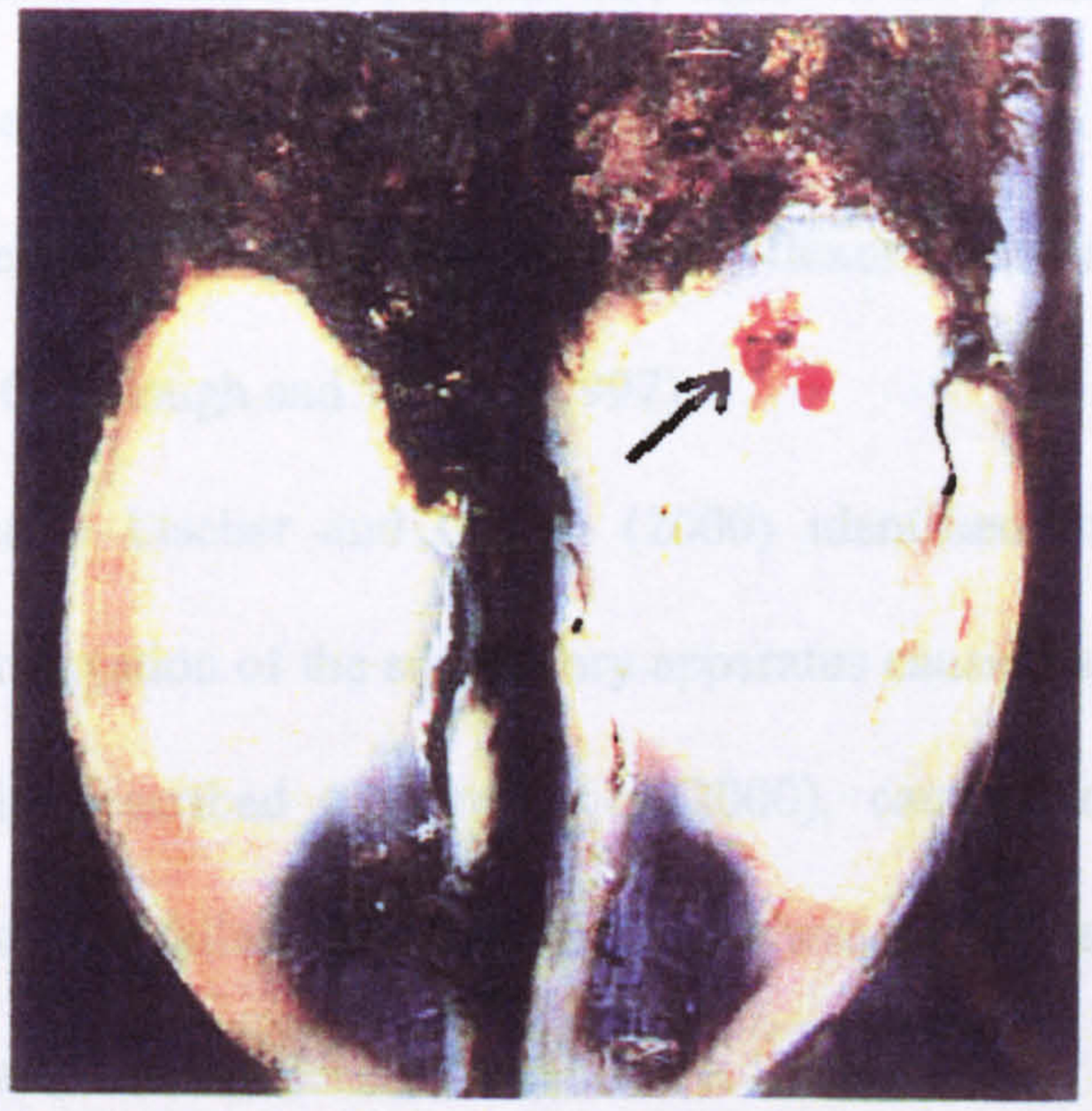


Plate 1.1: Site of the heel ulcer (taken from Blowey *et al* 2000).

The pathogenesis and aetiology of the sole ulcer can also be related to a newly identified lesion, the heel ulcer, which is described by Blowey *et al* (2000), except the heel ulcer's location lies under the third phalanx caudally to the sole ulcer site (Plate 1.1) (Blowey *et al* 2000).

Sole ulcers occur directly below the *Tuberositas flexoria* of the third phalanx. Opinions relating to the specific aetiology and pathogenesis have changed. Studies to date have identified that both biomechanical factors and disturbance to the claw corium vasculature damaging the stratum germinativum (as described in the previous section), occur prior to a sole ulcer incident and damage becomes visible at the sole surface as an ulcer or disrupted horn formation (Singh *et al* 1994b; Greenough and Weaver 1997; Lischer and Ossent 2000; Blowey *et al* 2000). External factors such as housing

environment and exostosis of the third phalanx also assist in the pathogenesis. The latter could be a result of healing and may contribute to a recurrence of the lesion (Greenough and Weaver 1997). In extreme cases the insertion of the flexor tendon and navicular bursa can become involved (Greenough and Weaver 1997).

However, recently Lischer and Ossent (2000) identified through histological studies that excessive relaxation of the suspensory apparatus causing sinkage of the distal phalanx, as previously described (Ossent *et al* 2000), caused sole ulcers, but on histological examination the failure of the laminar zone was not evident as the white line appeared macroscopically normal (Lischer *et al* 2000).

Lischer and Ossent (2000) also identified the presence of fat pads within the sole in three cylinder like structures located axially, abaxially and centrally with protruding fingers across the divides. The central fat pad is located over the *Tuberculum flexorium*. They proposed that this structure aided in the protection of the underlying structures and played a part in haemodynamics. They also found thinner sole corium, less fat pad content and more marked bone proliferation in the dorsal and lateral aspects of the distal phalanx in the cows that had sole ulcers (Lischer *et al* 2000).

The majority of sole ulcers occur in the weight bearing claws, lateral hind and medial front, which supports the influence of mechanical factors (Russell *et al* 1982; Clarkson *et al* 1996; Le Fevre *et al* 2001). Le Fevre *et al* (2001) also found a high correlation of sole ulcers found in both the lateral claws of the hind feet of cows, which implies that severe bilateral sole ulcers are common (Leach *et al* 1998).

Genetic correlations have also been observed between sole lesions and characteristics of claw shape, and an especially high genetic correlation was found in the measurements for the dorsal claw angle and toe length (Politiek *et al* 1986).

Sole ulcer occurrence has also been associated with reduced cow fertility, increasing days to conception, cull rates and service numbers (Collick *et al* 1989). Bergsten *et al* (1998) proposed that more frequent foot trimming reduced the potential severity of sole ulcers and this lesion may bear some relation to heel erosion occurrence.

White line lesion

Lesions in the white line have been described as white line separation, disease and abscess. 12-39% of lameness has been reported to be caused by white line lesions (Murray *et al* 1996; Greenough and Weaver 1997). It is considered to be one of the most common lesions observed in dairy cows (Baggott and Russell 1981), although seldom seen in some other countries such as Sweden (Bergsten *et al* 1998).

Due to its structure and function the white line is commonly observed to contain slight damage or debris in the oldest most distal surface, from the under-foot terrain (Baggott and Russell 1981; Greenough and Weaver 1997; Logue *et al* 2000). Some debris such as stones, if not dislodged, may penetrate further into the laminae leading to a potential infection that may travel to the corium and result in pain and disruption in the production of horn. Pain can also result from pressure within the foot which builds up from the establishment of an abscess (Baggott and Russell 1981).

An abscess if not attended builds up to create a separation between the horn and keratogenic layer in the wall or sole and with increasing pressure may finally discharge

through the coronary band or skin horn junction of the heel or penetrate the navicular bursa creating a septic arthritis (Blowey 1993; Greenough and Weaver 1997). In extreme and uncommon cases it can erode the *Tuberculum flexorium* of the third phalanx and create necrosis in the flexor tendon (Greenough and Weaver 1997).

The greatest number of haemorrhages and lesions in the white line are seen in zone 3 (Vermunt and Greenough 1996a; Logue *et al* 1998a) of the lateral hind claw (Baggott and Russell 1981; Blowey 1993; Greenough and Weaver 1997). Weight bearing occurs along the abaxial border of the foot, predominantly in the sole, around zone 3 (Figure 1.4) and the lateral hind claws and medial front (Vermunt and Greenough 1996a; Logue *et al* 1998c). Toussaint Raven (1985) also suggested that weight bearing is less even in the lateral hind than the medial, which is more even in distribution. All of these factors create stress within the white line and corresponds with the area where there is a high turnover of horn (Warzecha 1993).

Contributory factors to white line lesion include calving, increased horn moisture and factors that alter normal biomechanics and therefore the ability of the structure to withstand the forces in locomotion such as deformed claws, high body weight, overgrowth, hurrying, excess walking and sudden changes in direction (Baggott and Russell 1981; Greenough and Weaver 1997). Other factors include those which disrupt the internal production of the white line horn and leave low quality horn or horn containing necrosis or haemorrhage, such as vascular disturbance (Singh *et al* 1994b).

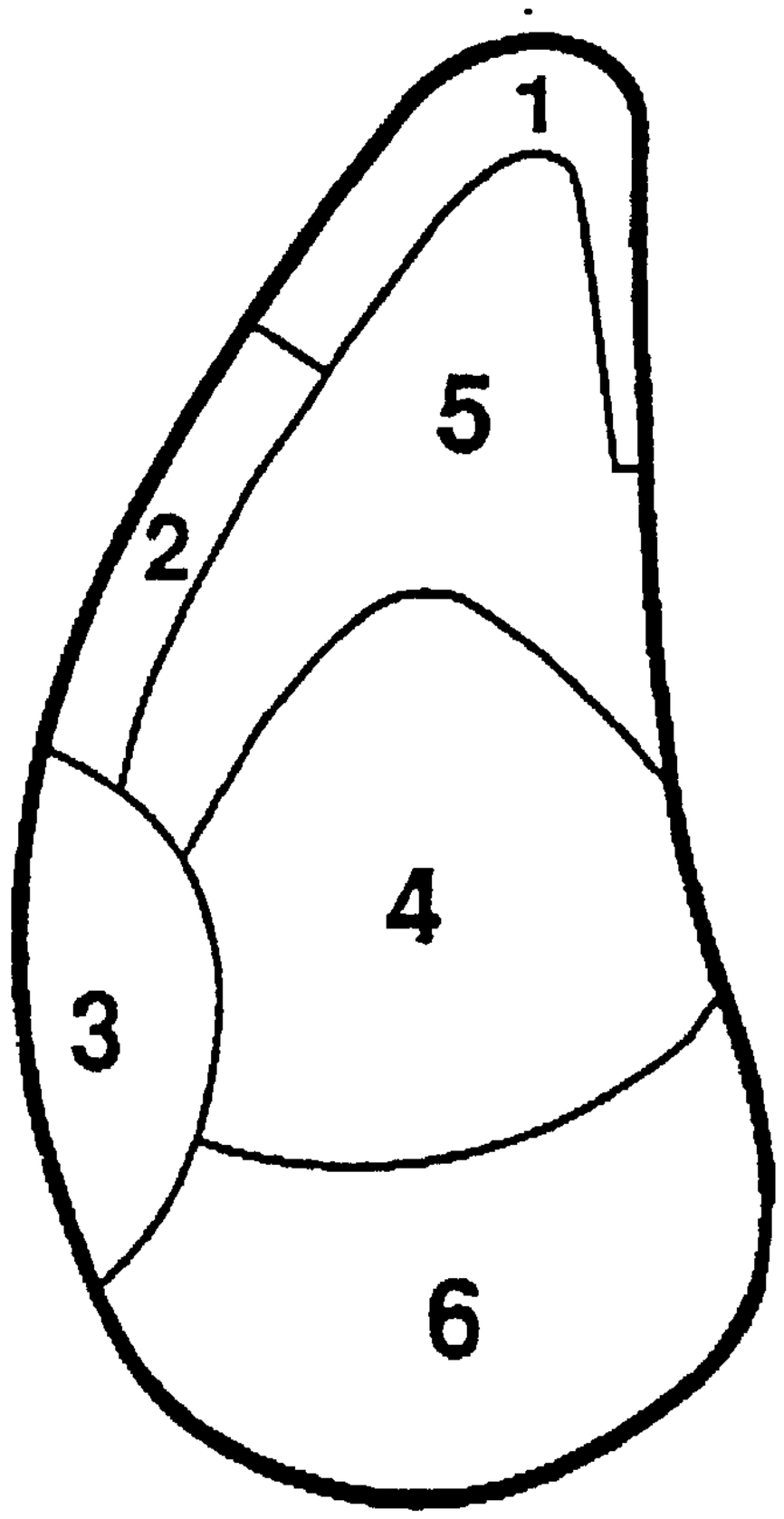


Figure 1.4: Zones of the sole. 1, white line at the toe; 2, abaxial white zone; 3, abaxial wall-bulb junction; 4, sole-bulb junction; 5, apex of the sole; 6, bulb of heel (Greenough and Vermunt 1991).

The theory that biomechanical loading is a risk is supported by the fact that its incidence is greater in countries or areas where longer grazing periods are adopted, which includes walking to milking parlours for example, the UK (22% taken from data on 19714 diagnosed lameness lesions idenitified within 120 days post calving) and Australia and New Zealand (40% taken from data on 427 diagnosed lameness lesions idenitified within 120 days post calving) (Logue *et al* 1998a; Fitzgerald *et al* 2000), although the management regimes vary greatly between different countries and therefore comparisons cannot easily be made.

Offer *et al* (1998), observed the peak lesion scores for white line lesion 16-26 weeks after calving and peak levels increased with increased parity of the animals within this scale. Baggott and Russell (1981) observed the lesion predominantly around 50 days and also 201-265 days after calving and during the winter months October to December. Logue *et al* (1998, 2000) suggested 10 weeks post calving for the lesion to appear and approximately 60% of white line and sole lesions can be identified in the 120 days after calving (Logue *et al* 1998a).

There is evidence that white line lesion occurs in older cows, Bergsten and Herlin (1996) found a high haemorrhage score in the white line in older cows in cubicles. Baggott and Russell (1981) also observed the greater number of white line lesions occurring in the claws of older housed cows. White line lesions are reported to be less common in cows less than three years old. Bargai and Mazrier (2000) studied primiparous and multiparous cows in 1998 and found that the multiparous cows had much more white line separation by 7 %, (26.8% (total of 338 cows) and 33.4% (total of 474 cows) respectively) which may have in this case been due to feed changes as a result of market prices.

White line lesions may occur sometimes bilaterally (Greenough and Weaver 1997; Le Fevre *et al* 2001). Leach *et al* (1998) found a significant correlation between the right and left hind lateral claws for white line lesion score. Bargai and Mazrier (2000) identified a shift associated with weight bearing from front medial claws in primiparous cows to hind lateral claws in multiparous cows (P 0.006).

Le Fevre *et al* (2001) found that severe lesions of the white line did not necessarily occur bilaterally. They also found that white line lesions were evenly distributed amongst

the eight claws of the cows, compared with sole ulcer, and commonly present in pairs of claws with the lowest prevalence of lesions. Another hypothesis may be that the cow alters her gait because of the pain which causes further lesion development (Leach *et al* 1997).

A relationship between sole ulcer and white line lesion has been postulated. However, Leach *et al* (1998) identified that any relationship was very loose and Le Fevre *et al* (2001) identified the importance of treating the lesions as separate entities (Logue *et al* 2000). Offer McNulty and Logue (2000) found that the incidence of white line and sole ulcer lesions peaked commonly in the same week in first lactation animals but became wider apart in later lactations. Although Leach *et al* (1997) observed the most severe cases of white line lesions in first lactation cows 9 weeks post calving simultaneous with housing, a sole lesion occurred 5 weeks later which suggests a difference in pathogenesis in the anatomical regions. It has been proposed however, that the laminar or white line horn displays damage in horn production, from the same origin, sooner than the sole region.

A white line lesion at the toe is less common (Baggott and Russell 1981; Greenough and Weaver 1997). It has been associated with congestion of the circumferential artery and the introduction of high energy feed. In some instances it has also been accompanied with a definite ridge in the wall material (Greenough and Weaver 1997). Vermunt and Greenough (1996a) observed a significant number of haemorrhages in this region in dry lot heifers. It is reported to be the site of the most severe form of white line damage and often requires more treatment and recuperation (Logue *et al* 1998a).

UNIVERSITY OF BRISTOL LIBRARY
MEDICAL

Sole haemorrhage

Sole haemorrhage is defined as the presence of blood in the sole of the claw. Like the sole ulcer, sole haemorrhages are located predominantly in the lateral hind and medial front claws, associated with weight bearing (Bergsten 1994; Bergsten and Herlin 1996; Vermunt and Greenough 1996a). Haemorrhages may accompany another lesion as well as being a primary lesion (Blowey 1993).

They may occur as a result of constriction in the terminal part of the digital artery in the sole ulcer site (Singh *et al* 1994b) and repeated insults or vasoactive episodes cause layers containing sole haemorrhages and scar tissue (double sole) (Brizzi *et al* 1998).

The most severe haemorrhages commonly occur around calving; this may be as a result of underlying physiological problems possibly associated with production stress rather than changes in diet which may exacerbate the process (Logue *et al* 1994). Midla *et al* (1998) found that mild sole haemorrhages were observed in only a few heifers 25 days post calving. Vermunt and Greenough (1996a) however, found sole haemorrhage in 77% of heifers at approximately 1 year of age (prior to calving).

The observation of sole haemorrhages in the claw has been indicated as an effective measurement to study the aetiology and pathogenesis of clinical and subclinical laminitis (Livesey and Fleming 1984; Bergsten 1994). Haemorrhage in first lactating heifers has been described as causing permanent damage to the claw and prevention of these lesions is therefore critical in preventing or reducing the frequency of lameness in future lactations (Bergsten 1994; Vermunt and Greenough 1996a; Logue *et al* 1998b).

Leach *et al* (1998) found heifer lateral hind claws free from haemorrhage 9 weeks post calving but the peak severity was 14 weeks post calving (Leach *et al* 1997). Logue *et al* (1994) identified lesion formation 3 months post calving in heifers. Livesey *et al* (1998a) also identified high scores for sole haemmorhage in early lactation. Bergsten and Herlin (1996) found that 94% of heifers and 66% of older cows had sole haemorrhages when examined at 2-4 months post calving.

Haemorrhage is proposed to take longer to dissipate in the older cow, locomotion scores remain high for longer and are more variable than heifers, lesion onset is also proposed to be less predictable than heifers and recurrence of injury to the claw corium is very detrimental (Greenough and Vermunt 1991; Logue *et al* 1994).

Axial groove fissure

Also termed as axial wall crack, axial groove crack and interdigital abscess, these lesions can occur in all claws on the axial aspect and run distally next to or along the axial groove of the claw. They are most common in the hind lateral claws. These lesions have been considered to have the same pathology as a white line lesion as the white line runs up inside the axial aspect of the claw (Blowey 1999, personal communication). It is the most common lesion requiring veterinary treatment in dairy cows in Australia and occurs occasionally in New Zealand (Vermunt 1998).

Vermunt (1998) observed this lesion in both corkscrew claws and claws considered normal and healthy. Like other lesions it is associated with poor hygiene and wet conditions, especially in the event of heavy rain in Australia.

Vertical wall fissure

This lesion is also known as a sand crack. It commonly occurs in the dorso-axial wall and sometimes, but very rarely, the abaxial wall running from the periople or coronary band along with the direction of growth of the wall, potentially running to the bearing surface. Complete cracks produce pain because the corium is exposed. Infection then finds its way to the corium and potentially deeper structures, and inflammation also occurs in the coronary band (Baggott and Russell 1981; Greenough and Weaver 1997). The lesion, when only slight, may go unnoticed if covered by manure and may not produce lameness. It has been more commonly identified in beef cattle, approximately 37% affected in Western Canadian beef cows (Campbell *et al* 1996), than dairy cows where it is fairly low in incidence, 0.5% in one study (Greenough and Weaver 1997). De Vecchis and Mortellaro (1998) observed, from trimming records, an incidence of 0.64% from 5601 dairy cows in Italy.

It is most commonly and historically associated with dry conditions as it may be a result of excessively dry horn cracking because it cannot expand sufficiently in response to an applied load. It has also been attributed to abnormal claw conformation or trauma damage to the coronet, which impedes the correct growth of coronary horn, or disruption in horn formation from incorrect nutrition (Baggott and Russell 1981; Greenough and Weaver 1997).

It occurs in the hind medial claws but more often in the lateral fore claws, which have the greatest area of ground contact and the fore claws have the greatest weight carriage approximately 60%. The 100 days before calving is considered to be the time at

which cows are at the greatest risk of producing a vertical wall fissure (Baggott and Russell 1981).

In the instance of complete large cracks intervention is required to close the gap as natural healing is impossible (Greenough and Weaver 1997).

Horizontal wall fissure

Often referred to as a hardship groove where there is depression around the circumference of the claw or a 'thimble' where this groove is ruptured. It originates from the coronary border and moves slowly down the wall with the production of further wall horn (Greenough and Weaver 1997; Brizzi *et al* 1998). The fissures are rare and are mainly observed in beef cattle 0.8%-2.05% (Greenough and Weaver 1997).

The groove is commonly associated with metabolic changes in the cow, for example calving and nutritional changes which effect the production and quality of horn at the coronary site (Greenough and Weaver 1997; Brizzi *et al* 1998). These are painless and when calculated with the rate of horn growth, allows an estimation of the time at which the stressful episode occurred (Greenough and Weaver 1997). With growth the groove becomes a fault line where potential damage can occur, more than one groove can alter the conformation of the claw (Brizzi *et al* 1998).

When a fissure travels down the claw it places great pressure on the lower claw, termed the thimble, and the internal claw, and causes a lot of pain. Usually all eight claws are affected similarly (Greenough and Weaver 1997).

Interdigital necrobacillosis

This lesion has several different names which include interdigital phlegmon, infectious pododermatitis, foot rot and foul in the foot. It occurs in the interdigital epidermis as a necrotic infection and is very painful, causing severe lameness. A common and consistently isolated organism from this lesion is *Fusobacterium necrophorum* subsp. *necrophorum* and *Porphyromonas levii* which is an anaerobic bacterium present in the rumen and to a lesser extent in the faeces, and responsible for the foul smell (Berg and Franklin 2000).

Interdigital necrobacillosis is an infectious condition which spreads rapidly in the soft tissues of the skin, although the bacteria are not known to have an invasive ability so infection requires an initial insult (Baggott and Russell 1981; Greenough and Weaver 1997; Berg and Franklin 2000). Spirochaetes have also been identified in some lesions that looked like interdigital necrobacillosis and may suggest a relationship with digital dermatitis (Dopfer 2000 cited Doherty *et al* 1998).

The lesion arises from trauma to the skin of the claw; internal systemic problems to a physical blow, potentially from a dry environment, uneven ground, rough terrain or damage from excess water, urine or faecal material (Baggott and Russell 1981; Greenough and Weaver 1997).

Interdigital necrobacillosis consists of inflammation of the skin evenly around the interdigital space and coronary band which may extend up the leg and push the claws further apart than normal (Greenough and Weaver 1997). The body temperature of the animal also increases and appetite and milk yield drop (Baggott and Russell 1981).

If the condition continues, an exudate becomes evident followed by necrosis of the tissues involved (Baggott and Russell 1981). In severe cases problems with the distal interphalangeal joint, navicula bursa, flexor tendon and a retroarticular arthritis may occur. Occasionally septicaemia and toxaemia ensues (Greenough and Weaver 1997).

Kossaibati and Esslemont (2000) reported 36% of total skin lesions causing lameness were interdigital necrobacillosis in 50 herds in southern UK. An interdigital necrobacillosis lesion incidence of 15% which reduced significantly in herds with greater than 200 cows, was observed by Rowlands *et al* (1983). Parizi and Hosseini (1998) found overall 60% interdigital necrobacillosis in six herds of cows in Shiraz, Iran. Baggott and Russell (1981) found it to be the second most common lesion that required veterinary treatment after white line disease.

Baggott and Russell (1981) reported that the majority of independent cases of interdigital necrobacillosis were observed in the first 50 days after calving, but cases linked with other lesions were most prominently observed 201 to 265 days after calving. Alban *et al* (1995) also reported a positive association between other lameness lesions and the incidence of interdigital necrobacillosis (Baggott and Russell 1981 and Greenough and Weaver 1997).

It is observed more commonly in the hind claws but may be seen in the fore feet. It usually only effects one foot but has been seen in all feet of calves (Baggott and Russell 1981; Greenough and Weaver 1997).

Individual immunity and temporarily acquired immunity (calves), influences the presence of this lesion. (Baggott and Russell 1981 and Greenough and Weaver 1997). Therefore older cows are less susceptible and adults of 3 to 8 years are the greatest risk

group (Baggott and Russell 1981). Alban (1995) however, found that second lactation Danish dairy cows had the lowest risk of having this lesion and first lactation cows were at greatest risk.

Foot trimming is proposed to reduce the frequency of interdigital necrobacillosis although many infected claws are a normal shape (Greenough and Weaver 1997).

There has also been a new version of this lesion identified more recently which is termed 'super foul' which is most common in dairy cows. This lesion starts with a severe swelling which progresses rapidly into a severe deep tissue lesion with severe necrosis and is very difficult to treat and cure (Blowey *et al* 1994; Greenough and Weaver 1997; Berg and Franklin 2000).

Digital dermatitis

This lesion was identified and classified during the 1970's, and first reported in the UK in 1987 (Blowey *et al* 1994). It spreads easily within a herd and is very painful. Interdigital dermatitis and digital dermatitis are said to be associated, although interdigital dermatitis is an ill-defined lesion (Blowey 1993; Blowey *et al* 1994). Different infective agents have been identified but the macroscopic evidence that belongs exclusively to interdigital dermatitis is very difficult to find (Greenough and Weaver 1997; Dopfer and Willemen 1998). Read *et al* (1998), reported histopathological and immunohistochemical evidence that papillomatous digital dermatitis and digital dermatitis belong to the same disease complex. Information for infectious skin lesions of the claw still remains largely unstandardised and unestablished (Dopfer and Willemen 1998; Bargai 1998; Dopfer 2000).

Digital dermatitis involves the production of raw granulation of the epidermis, which produces exudate. It is commonly observed proximal to the interdigital space cranial and caudally. It can take the form of an erosive or reactive lesion, which is purulent and smelly, consisting of granulation tissue with a few superficial hairs, but can also be a proliferative wart type lesion which consists of hard tendrils, which is proposed to be epithelial proliferation which develops in an attempt to shed the organisms (Blowey 1993; Blowey *et al* 1994; Greenough and Weaver 1997). Budras and Mulling (2000) proposed that intraepithelial nerves that are responsible for pain information may provide chemical signals to keratinocytes for cell proliferation which may also adapt epidermal structure to changed mechanical loading.

Spirochetes (several of the *Treponema spp*) with bacteria or viruses help develop the pathogenesis and it is proposed that the lesion develops as a result of the environment, age and immunity of the cows (Blowey *et al* 1994; Greenough and Weaver 1997; Dopfer 2000). Identification of specific spirochetes has been made, which are proposed to have a predilection to keratins of the epithelium and a keratolytic function through toxin (Blowey *et al* 1994). Reproduction of the disease artificially has proved very difficult and is therefore attributed to more than an infectious element (Greenough and Weaver 1997; Read and Walker 1998b).

The lesion tends to be either present in a number of the cows or absent from the herd and this supports the infective nature of this lesion. Kossaibati and Esslemont (2000) observed 56% of cows affected with digital dermatitis of the total skin lesions in a DAISY study on 50 herds in the south UK.

Digital dermatitis is often picked up by heifers (Rodriguez-Lainz *et al* 1999) as they first enter the herd also implying an immunological factor. First parity cows late in lactation are also proposed to be at greater risk of contracting digital dermatitis (Dopfer 2000). Argaez-Rodriguez *et al* (1997) and Dopfer (2000) suggest that purchased animals were 3.4 times more likely to contract the disease than the animals born on farm. Rodriguez-Lainz *et al* (1999) found a dose effect decrease in digital dermatitis with increasing lactations and an increase in odds with days in lactation. Argaez-Rodriguez *et al* (1997) also found that the calving to conception days were significantly extended in cows with digital dermatitis.

Interrupted horn growth may result when the lesion occurs directly adjacent to the site of horn production, and can be a predisposing factor of heel horn erosion or vertical wall fissure. It has also been known to cause sole ulcers in extreme cases where the corium has been effected (Greenough and Weaver 1997).

The use of foot-baths reduces incidence of digital dermatitis (Blowey *et al* 1994; Dopfer 2000).

Foreign body

A lesion caused by a foreign body is sporadic and often immediately very painful and purulent material gathers rapidly (Baggott and Russell 1981; Greenough and Weaver 1997). The degree of lameness is very variable (Baggott and Russell 1981).

It occurs in the form of penetration or just pressure from foreign bodies such as stones, splinters of wood or glass or any sharp object. Foreign bodies may be stepped on

and remain in the foot, or fall away immediately, or soon after. Pressure from the foreign body can cause local necrosis in the underlying corium which develops an abscess (Greenough and Weaver 1997). Penetration depends greatly on condition or quality of the claw for example, over-worn soles or wet or humid conditions causing soft horn, the surface on which the animals walk and how far and fast they walk. Rough hard tracks or hard surfaces are high risk factors (Baggott and Russell 1981; Blowey 1993; Greenough and Weaver 1997). Therefore the time of greatest risk are the summer months when all of these factors are most prominent (Baggott and Russell 1981).

Eddy and Scott (1980) found 4.8% of lame cows with foreign bodies, 20.4% had pricked soles, 1.2% of lameness was attributed to over worn soles and 12.7% bruised soles. Kossaibati and Esslemont (2000) observed 31% of all lame cows had foreign body penetration. Baggott and Russell (1981) report that sole foreign body penetration occurs mostly in medial fore feet and represented 3% of lesions. In extreme cases deep penetration around the heel sole junction may involve the *Tuberculum flexorium,* the navicular bursae or the pedal bone.

Slurry heel

This is also called heel horn erosion or *ersio ungulae* and defined by loss of the structure of the heel bulb and a marked pitting or oblique grooves (Baggott and Russell 1981; Greenough and Weaver 1997). It has also been implicated as a result of digital dermatitis (Peterse 1985; Blowey 1993) because the bacteria identified in cases of digital dermatitis have been present (Greenough and Weaver 1997). Although Sweden has heel horn erosion but apparently no evidence of digital dermatitis exists (Bergsten *et al* 1998).

This lesion is commonly observed in animals in wet and unhygienic conditions, so it is often seen in the winter months from January to June. Mild erosions are commonly observed in dairy cow heels and can predispose the heel to complications (Baggott and Russell 1981; Greenough and Weaver 1997). In mild cases this lesion does not often create lameness (Baggott and Russell 1981).

Severe damage is observed as black fissures in the epidermis that continue across and involve the heel horn and these fissures always run in a horizontal plane around the back of the claw (Baggott and Russell 1981; Greenough and Weaver 1997). Sometimes compensatory horn develops on the sole just below the heel which causes an increase in pressure on the corium as the distribution of weight is altered (Greenough and Weaver 1997).

Choquette-Levy *et al* (1985) reported that 11.8% of lesions observed were heel horn erosions. Enevoldson *et al* (1991a) identified 44% heel horn erosion in first lactation cows and 69% in later lactations with increased severity and an increased risk with stage of lactation (Enevoldsen *et al* 1990; Offer, McNulty and Logue 2000). Livesey *et al* (1998a) identified an increase in lesion score for slurry heel in the weeks post calving.

Overgrown claws are a risk factor for slurry heel (Baggott and Russell 1981) and Bergsten and Herlin (1996) observed this lesion predominantly in the hind feet.

Severe sole ulcer lesions have also been identified as a risk factor for heel horn erosion and interdigital dermatitis (Enevoldsen 1990). It may be that these lesions happen to be present at the same time rather than being the cause of a secondary lesion, because they are all associated with slurry contamination.

Heel horn erosion has been reduced with the introduction of a footbath (Bergsten and Herlin 1996).

Under run sole or wall

This lesion has a similar aetiology and pathogenesis to heel horn erosion. As its name suggests, it consists of a necrosis that runs under the bearing surface of the sole or under the wall. Under run sole is often considered a double sole although the under run sole separates in the heel region (Brizzi *et al* 1998). Under-running may be associated with other lesions such as sole ulcer or white line separation where the necrotic infection spreads into these areas. The main days at risk are 50 to 150 days after calving when the lesion is associated or secondary to another lesion. As a lesion itself the risk days are up to 50 days after calving (Baggott and Russell 1981).

Like other lesions, the main factors that predispose to these lesions are wet and unhygienic conditions which help to promote anaerobic conditions and bacteria and poor quality horn or horn damage (Baggott and Russell 1981).

Interdigital growth

Known also as interdigital skin hyperplasia, granulomas, wart, tyloma and other similar names. It is identified by a hard mass of inflamed skin of variable size located between the digits, often with pressure necrosis. This occurs as a result of a proliferative reaction causing hyperkeratosis, parakeratosis and secondary damage often including infection. Splayed feet can also be a predisposing cause (Baggott and Russell 1981). It is not really sensitive to pain. It occurs as a result of irritation or dermatitis from a previous

lesion such as interdigital dermatitis, or faecal irritation caused by dried material in-between the claws, which forces the claws and underlying structures apart. It is proposed to originate from the axial side of the lateral digit. Severe cases involve purulent material and ulceration (Baggott and Russell 1981; Greenough and Weaver 1997).

It is observed more commonly in the hind claws than the fore claws and can be quadri, uni, or bilateral. Quadri and bilateral lesions observed in animals younger than 2 years imply a potential heritable factor (Baggott and Russell 1981; Greenough and Weaver 1997).

Interdigital growth has been found to be responsible for 4.2% (Russell *et al* 1982) and 6% incidence (Enevoldon *et al* 1991) and in first lactation cows 1% incidence (Enevoldon *et al* 1991). Occurring predominantly in overgrown feet and in larger herds (Baggott and Russell 1981; Greenough and Weaver 1997).

The degree of lameness observed depends greatly on the lesion size, as some small lesions remain for years and other larger, purulent lesions cause necrosis and locomotion problems and pain and therefore subsequent loss of condition. There is also a risk of trauma to the lesion itself (Baggott and Russell 1981; Greenough and Weaver 1997).

Lameness assessment

The term lameness score was introduced by Manson and Leaver (1988a) who initiated the first method of measuring the extent of lameness in an individual animal. Scoring is carried out commonly to assess prevalence, severity, and duration of lameness. As a potentially hugely variable objective measurement, experimental repeatability of

lameness scores is shown to be surprisingly quite high, although one observer was involved in the study by Manson and Leaver (1988a). As previously described, Murray *et al* (1994) suggested that for large scale surveys it would be better to employ several trained observers to achieve the amount of data required than restrict the number of sites or observers used, because an accuracy of 80% was observed with one or more trained observers. Eddy and Scott (1980) or Russell *et al* (1982) did not evaluate observer consistency or train and in these cases data were taken from a wide range of sources.

Cows normally only change their pattern of locomotion or walking if they are experiencing pain or discomfort. However, the cow may be lame or possibly stiff or momentarily experiencing pain caused by an environmental factor such as a foreign body (stone) and these need to be segregated when reported. In some situations it may be disadvantageous for a cow to display its pain. Although Whay *et al* (1997) found interactions between lameness, claw lesions and the development of hyperalgesia.

Method of lameness assessment - Factors that help to minimise incorrect diagnosis include; unstressed conditions, conformational differences acknowledged, age, flooring. Some cows may be stiff due to confinement.

Observations in locomotion include: a symmetrical arc of digital flight, equal weight bearing, straight limb progression line, even, smooth and rhythmical gait between swing and stance and retraction and protraction (Greenough and Weaver 1997).

Locomotion score has been considered as a representative measure of an individual's life productivity or survival within the herd. A significant effect of locomotion score with weekly milk yield has also been observed (Logue *et al* 1994).

Determinants of lameness - epidemiological and experimental studies

Causes of lameness in different species have been the subject of many research papers. Lameness, mainly the claw, has reached a point where it is secondary only to infertility as a major reason for involuntary culling of dairy cows, with similar levels to those for mastitis (Distl 1995; Boettcher *et al* 1998). Mastitis, metritis, teat injuries, infertility, poor milk yield are some of the primary risk factors for (cause), or may be secondary (consequence) to lameness (Peterse 1985; Mortensen 1994; Ossent and Lischer 1994; Peeler *et al* 1994) Other indirect factors include diseases such as lactic acidosis, fatty liver syndrome and possibly resultant endotoxaemia (Peeler *et al* 1994).

Potential causes of lameness can be divided crudely into genetic, conformation and breed influences, nutritional influences, calving, management effects and environmental factors. These cover a very broad range and it may not be one factor that causes lameness but a variety of combinations of these factors. These factors are discussed in the following section.

Conformation

Conformation of the cow and specifically the claws and body weight can effect the shock absorbing capabilities of the horn (Baggott and Russell 1981). Not surprisingly, posture and gait are also factors considered important and related to claw morphology and physiology and may predispose an animal to certain digital diseases. Abnormal posture may be inherent or acquired by an animal after birth (Distl and Mair 1990).

Extreme overload of the claw may be associated with the larger framed, heavy animals with straight pelvic limbs and small claws or in part claw conformation

(Greenough and Weaver 1997; Boettcher *et al* 1998; Distl and Mair 1990). Distl and Mair (1990) considered that morphological functional claw parameters did not have a close relationship with digital disease. Vermunt and Greenough (1996b) however suggested that morphological claw parameters were related to the frequency of claw disease in later life, which questions whether claw shape predisposes or is a result of claw disorders.

Commonly, the morphology and internal aspects of the medial and lateral claws are reported to be different (Ossent *et al*, 1987, Toussaint Raven 1973). Differences may be observed between the front and hind claws at late pregnancy despite the fact that load is reported to be even around calving (Bergsten 1994). An increased growth and therefore length in the front medial and hind lateral claws opposed to the opposite claws has also been observed (Andersson and Lundstrom 1981; Hahn *et al* 1984; Murphy and Hannan 1986). The lateral hind claw is also wider and the distal phalanx rougher than in the medial claw so the forces are therefore different in weight bearing (Ossent *et al* 1987; Toussaint Raven 1973).

Genetics and breed

The following conformational traits are proposed to be heritable factors; foot angle (decreased angle associated with clinical lameness; low negative correlation with first lactation yield), hoof length (phenotypic relationship with fat and milk yield and negatively related with survival), shallow heels (negatively related with survival) and deeper heels (positively related genetically to days in milk), rear legs or rear view (hocks in/toes out - more prone to clinical lameness), rump width (larger/wider and therefore

usually heavier more predisposed to clinical lameness) and stature (wither height) (Greenough and Weaver 1997; Boettcher *et al* 1998).

Selection criteria in young cows and bulls through claw measurements and hardness, locomotion and leg conformation, forces in the hoof at the ground surface floor interaction, have all been suggested for future breeding programmes (Russell 1986; Vermunt and Greenough 1995b; Distl 1995; Distl 1998). Logue *et al* (1998) and Choi and MacDaniel (1993) found that locomotion score and hoof measurement aided in identifying the significance of hoof angle as the only consistently significant trait influencing lameness and longevity of the cow, and also correlated to the genetic value of sires. Hoof measures and economic traits appear to be sufficiently high to be useful in selection for improved economic value and heritabilities of hoof traits for later lactations, which are higher than those for earlier lactations (Choi and MacDaniel 1993).

However, if claw parameters were to be taken to predict the future survival of the cows, measurement should be taken at 12 months of age before their first foot trimming and before calving, as these factors could effect the future morphology of the claw and affect possible judgment. Essentially early life assessment or measurements will alter with age (Vermunt and Greenough 1996b).

It is proposed by some workers that breed of the cow may predispose them to hoof diseases (Stanek and Stur, 1984 cited by Warzecha 1993) such as sole lesions (Bergsten 1994; Logue *et al* 1994), interdigital necrobacillosis lesion (Baggott and Russell 1981; Alban *et al* 1995; Greenough and Weaver 1997) and interdigital growth (Baggott and Russell 1981; Greenough and Weaver 1997). Dairy cows in the UK are commonly Holsteins and Friesians, which are reported to more susceptible to lameness (Maclean

1971; Andersson and Lundstrom 1981). Distl (1998) found that Jersey cows had the lowest disease incidence and the highest genetic correlation between conformation traits in cows and measurement in bulls.

It is believed that some claw disorders have a partially genetic basis related to claw parameters but of course measurements can be affected by the environment and management factors. A significant difference has been observed between cow 'type' and a diet by breed interaction for locomotion score and milk yield. Nutrition and genotype interacted to influence mobility, lesions of the hoof and lameness, however, the mechanisms for this were unclear (Logue *et al* 1994).

Management and environment

Claw trimming - Distl and Mair (1990) concluded that correct claw conformation in the 1st lactation was due to age, and with the aid of the correct weight distribution through corrective claw trimming in the mature animal, a more equal weight and footprint area distribution would be observed, and potentially less lameness (Manson and Leaver 1989). Offer, McNulty and Logue (2000) identified a significant weak post-calving effect on claw conformation. Trimming also has the potential to increase the static friction of the claw in young cows and therefore may reduce slipping (Phillips *et al* 2000). Phillips *et al* (2000) found that Belgian Blue cattle had a higher static friction although this was thought to be due to chemical composition of the claw, otherwise, no relationship was observed between conformation or breed and static friction. Bergsten *et al* (1998) also identified that foot trimming in Swedish cattle reduced the frequency of the most common lesions.

UNIVERSITY OF BRISTOL LIBRARY

Housing - Other environment and management factors influencing lameness include; type of housing, weather, bedding, slurry, movement or transport of cows, loafing space, social hierarchy and foot bath use (Distl and Mair 1990).

A considerable difference between cows that are housed indoors to those kept outdoors has been observed (Vermunt and Greenough 1996a,b) and changes in herd management can influence lesion prevalence (Baggott and Russell 1981; Smilie *et al* 1999).

Confinement in winter housing is often on hard surfaces such as concrete, which increases the likelihood of ischaemic disturbances in the circulation of the hoof, wear exceeding growth and therefore morphological claw changes such as steeper shorter claws (Greenough 1990; Bergsten 1994; Singh *et al* 1994b; Vermunt and Greenough 1995b; Faull *et al* 1996). Also lesions such as sole ulcers which are also commonly associated with high yielding cows (Arkins 1981; Choquette-Levy *et al*, 1985; Russell *et al*, 1982). Over-exercise on hard surfaces can also cause trauma, lameness.

Cubicle housing is reported to have a significant and potentially irreversible effect on lameness (Bergsten and Herlin 1996; Faull *et al* 1996; Kerr 1998; Meyer and Galbraith 1998) depending on the number of cubicles to number of cows and the measurement (length and width) of the actual cubicles for behaviour, position and comfort. Increased standing means an increased risk of sole ulcer lesion (Baggott and Russell 1981; Russell *et al* 1982; Colam-Ainsworth *et al* 1989; Singh *et al* 1993). Cubicle housing also increases the risk of white line disease (also observed at pasture) (Livesey *et al* 1998a) and sole haemorrhages in both cows and heifers (Bergsten 1994; Bergsten and Frank 1996; Vermunt and Greenough 1996a; Livesey *et al* 1998a).

During cubicle housing lame cows have been observed to have significantly smaller dorsal claw angle than non-lame cows (Meyer and Galbraith 1998), attributable to a higher horn turnover with a slight excess wear rate, which has been seen in heifers reared in cubicles (Livesey *et al* 2000a). Although toe length has also been reported to increase with cubicle housing (Vermunt and Greenough 1995b).

Heifers in cubicles can experience thinner heels than straw yarded animals (Livesey *et al* 2000a; Webster 2000) Permanent straw yarded heifers may experience lower horn turnover and necessary foot trimming, lower heels, potentially thinner soles, unsuitable claw conformation development and an increased prevalence of sole haemorrhages in the hind lateral claws, particularly zones 3 and 4 (Livesey *et al* 2000a).

Communal housing also promotes lesions such as digital dermatitis, slurry heel (Livesey *et al* 1998a) interdigital necrobacillosis (Baggott and Russell 1981; Alban *et al* 1995; Greenough and Weaver 1997), commonly associated with poor hygiene and rapid spread, especially loose-housed (Greenough and Weaver 1997; Rodriguez-Lainz *et al* 1999; Dopfer 2000).

Although interdigital necrobacillosis and white line does not have a predilection to management system, housed or pastured (Berg and Franklin 2000). Bergsten and Herlin (1996) overall observed heel horn erosion predominantly in the hind feet than the for feet, although cows in cubicles had higher heel horn erosion in fore feet than the hind feet.

Vertical wall fissures are more likely to be observed in cows at pasture rather than those in loose housing (Baggott and Russell 1981).

Herd behaviour - Colam-Ainsworth *et al* (1989) identified that lying down was an important behaviour for dairy cows and that cows in high lameness incidence herds spend

a lot of their time standing. Standing reduces normal blood flow within the foot which is reliant on exercise for normal circulation. Confinement in winter housing plays a major part (Greenough 1990; Bergsten 1994; Singh *et al* 1994b; Faull *et al* 1996), cows at pasture are also observed to lie down less frequently but lying time is longer (Singh *et al* 1993). Spring calvers lying down times are significantly shorter than autumn calving cows (Berry *et al* 1998b; Chaplin *et al* 1998). Other factors include yarding time, the dominance hierarchy and some lesions, for example Berry *et al* (1998b) found a significant negative effect of digital dermatitis on lying down time and a significant positive effect on total lying down time. Therefore, cows with digital dermatitis did not lye down as often as other cows but when they did it was for a longer period of time, which may be indicative of the pain involved in this lesion.

Heifers are commonly the lowest in the dominance heirarchy and in first lactation they lie down less when they first enter housing than later in their lactation (Singh *et al* 1993). Separating first calving heifers can reduce slightly the incidence and prevalence of lameness (Logue *et al* 1998c).

Lying has also been associated with lesion incidence, such as sole ulcer which is related to the frequency of lying (Baggott and Russell 1981; Colam-Ainsworth *et al* 1989; Singh *et al* 1993).

However, Berry *et al* (1998a) found no significant effect of lying time or standing behaviour on overall foot lesions however only 52 animals were observed.

Environmental conditions - Poor management or environment can cause possible changes in milk yield, increased calving interval, and poor body condition (Colam-Ainsworth *et al* 1989; Andersen and Jarlov 1990; Greenough 1990; Weaver 1990;

Bergsten 1994; Singh *et al* 1994a; Faull *et al* 1996; Greenough and Weaver 1997; Budras *et al* 1998a,b; Logue *et al* 1998a,b,c; Mulling and Budras 1998; Meyer and Galbraith 1998) and may manifest in depleted adrenal function and aggravated rumen acidosis and other conditions, including laminitis, associated with the release of prostaglandin inflammation mediators (Andersen and Jarlov 1990).

The affect of slurry on horn samples was investigated by Mulling and Budras (1998) who observed structural alterations in healthy and lame cow's hoof horn, where samples were incubated in manure and urine solutions at room temperature. Manure extracted the majority of the intercellular cementing substance in the horn when examined under electron microscope, whereas urine altered the structure of the horn cells (keratin protein removal) and not the intercellular cementing substance. Ultrastructurally the keratin filaments diminished and the structure became sponge-like. Budras *et al* (1998b) observed the same in horses. Wet conditions can also effect the shock absorbing capabilities of the horn (Baggott and Russell 1981).

Many lesions can be observed commonly in wet or moist conditions, often associated with the winter months as previously detailed, such as digital dermatitis, although it has be found increasingly at dry pasture (Baggott and Russell 1981).

Growth and wear - Studies have investigated claw growth and wear (ideally occurring at equal rates), hardness and softness, susceptibility to lesions or overall quality. Horn growth alters at different stages through the lactation of the individual cow and relates some what to the metabolic process, breed, age, wear, season and the supply of essential nutrients (Tranter and Morris 1992; Vermunt and Greenough 1995b and 1996b; Livesey *et al* 1998b). Footbathing however reduces the moisture content of the claw and

therefore increases the hardness, which may allow growth to exceed wear (Vermunt and Greenough 1995b).

Sole wear occurred most rapidly along the abaxial edge of the weight-bearing surface, less quickly in the toe and heel areas, and most slowly in the mid-sole region. Both hoof wall wear and sole wear are greater in lateral digits than in medial digits of the hind feet and especially at housing, where maximal values for wear in lateral claws and minimum for medial claws were observed in first lactation heifers (Tranter and Morris 1992; Offer, Logue and Leach 2000; Livesey *et al* 2000a).

Contradictory views have been expressed with regard to the effects of season on claw keratinisation or growth. Many relate the increasing day length and warm summer months to increased growth because of increased circulatory assistance in the warm environment (Tranter and Morris 1992). Wear however depends on the housing or field conditions such as ice or soft earth which results in a decreased claw wear. Claw overgrowth can also indicate accelerated growth which has been associated with laminitis (Vermunt and Greenough 1995b).

Surprisingly cows on straw have a greater wear of the dorsal border (4.6mm per month) than cubicle housed cows (3.0mm per month) although these cows did have a non-significant higher claw growth. Wear is also greater after turnout ($P<0.01$) (Meyer and Galbraith 1998).

Mineral links have been suggested as an influencing factor in growth and wear: harder keratin was associated with higher concentrations of calcium, phosphorus, copper and zinc and significantly lower concentrations of water, sodium, potassium and iron and varied greatly with horn position within the claw and observation of lameness. This may

suggest a difference in availability of these elements for the formation of keratin cells (Baggott *et al* 1988).

Accelerated growth may occur as a result of hyperplasia of the keratogenic layer from an insult and subsequent damage, commonly in the lateral hind, with more mature cows possibly developing an uneven weight distribution through the hind claws as a result (Toussaint Raven 1985).

Track surfaces - Track surfaces also pose a large lameness risk. Movement or transport of animals may involve walking to and from pasture, which can involve passing over a short or very long distance over varying surfaces. This increases horn wear and potential foreign body penetration, discussed earlier (Meyer and Galbraith 1998) and maintenance of a good quality and well designed track is of huge importance, especially where grazing is carried out for all or most of the year. Length and quality of the track, is design and the collecting yard's abrasiveness and the amount of time located in the yard are all key risk factors (Harris *et al* 1988; Chesterton *et al* 1989; Clackson *et al* 1991; Tranter *et al* 1991; Vermunt 1992). Areas of high traffic density are also thought to become infected with organisms associated with digital diseases (Greenough and Weaver 1997).

Pasture maintenance - Pastures which are heavily manured or treated with nitrogenous fertilizers can increase the risk of nitrate toxicity, therefore posing a possible indirect risk on lameness through vasodilation and endothelial damage (Greenough and Weaver 1997; Vermunt 2000).

Calving

Calving influences horn production pre- and post-calving in heifers and cows (Kempson and Logue 1993). This includes influences from management and other changes such as sudden changes in hormones (Bergsten 1995), surroundings (grazing to housing), diet and herd dominance. A significant interaction between diet and housing has been observed at calving (Bergsten and Frank 1996; Livesey *et al* 1998b), although metabolic demands in autumn calvers are lower than those for spring calvers, who lose more weight after calving (Berry *et al* 1998b; Chaplin *et al* 1998).

Nutrition is discussed in a later chapter, although nutritional deficits occur in late pregnancy and early lactation as a result of calving stresses and horn growth also slows at this time (Chamberlain and Wilkinson 1996; Livesey *et al* 1998b). As heifers are still maturing at the stage of their first calving and lactation it is proposed that they are at greater risk of lameness at this time of deficit (Livesey *et al* 1998b).

Hormonal changes that occur during pregnancy and lactation may play a key role. Epidermal growth factor (EGF) (indicated in both the equine and bovine hoof) may create a possible physiological and regulatory effect on hoof epidermal keratinisation (Hendry *et al* 1997) and exogenous hormones such as prolactin, a key lactogenic hormone in ruminants that decrease protein synthesis of the bovine hoof (Hendry *et al* 1997). Other hormones that may affect hoof epidermal keratinisation include glucocorticoid (higher levels in high yielding cattle), and insulin (which may relate to the predisposition of laminitis, where a high energy feeding regime is in place, and overfeeding in the dry period). Therefore, this appears to provide evidence for an endocrine basis for the increased susceptibility of lactating animals to lameness (Hendry *et al* 1997), although

studies are still largely inconclusive. Holah *et al* (2000) highlighted the possible effects of the hormone relaxin, which is responsible for relaxation of fibrous tissues at parturition, and questioned whether it may relax other tissues including hoof fibrous tissue at this time, such as the suspensory apparatus as previously discussed (Ossent *et al* 2000).

Damage to horn following calving commonly appears in the form of haemorrhage in the white line and sole approximately 10 weeks post calving (Logue *et al* 1998a; Logue *et al* 2000). More sole haemorrhages and digital dermatitis (Greenough and Weaver 1997; Rodriguez-Lainz *et al* 1999; Dopfer 2000) are also observed in autumn calving than spring calving heifers. Bergsten and Frank (1996) suggested that this could be related to sudden housing and the spring calvers may be used to the housing. Sole haemorrhages after calving were also significantly correlated with the ultrastructure and mobility or locomotion score of the individual (Kempson and Logue 1993). Enevoldson *et al* (1991b) also found a high positive association between high yield in early lactation and high body weight to sole ulcer occurrence.

Nutrition

Nutritional factors of the dairy cow have been implicated as one of the most important risks to lameness. This includes the level of concentrate fed, frequency of feeding or the length of time over which changes in feeding around calving occur (Manson and Leaver 1988a + b).

Pre- and post-calving concentrate rations increase, sometimes suddenly, resulting in high concentrate:forage ratios which are implicated as a causal factor for poorer quality, softer horn being more susceptible to trauma and therefore lesions like sole

haemorrhage, sole ulcers and white line (Livesey and Flemming 1984; Manson and Leaver 1989; Bergsten 1994; Coulon *et al* 1995; Livesey *et al* 1998a; Bargai and Mazrier 2000). Some workers have considered that the level and composition and quality of the concentrate may be more important as a risk factor for lameness than the adaptation period to the diet (Peterse 1985). Others have also considered that the diet offered pre-calving did not affect lameness during lactation (Leaver 1990). Sudden changes in ration composition can occur as a result of economic pressures and turnout onto grass. These are also risk factors for other diseases which are commonly linked to lameness, (Manson and Merritt 1990; Vermunt and Greenough 1990; Greenough and Weaver 1997; Bergsten and Frank 1996; Livesey *et al* 1998a).

Carbohydrates (readily fermentable sugars or starch) fed in high rates, especially to unconditioned cows, can increase the risk of lactic acid build up in the rumen which reduces the pH and therefore changes the micro-flora. Changing the bacteria from gram-negative organisms to gram-positive involves bacteriolysis, which involves the release of endotoxins into the system, and vaso-active substances identified as causing laminitis challenge in the claw when produced at sufficient levels (Peterse 1985; Blowey 1993; Da Costa Gomez *et al* 1998; Seymour 1998; Vermunt 2000).

High protein rather than energy has also been implicated as a factor that increases prevalence of laminitis associated lesions (Manson and Leaver 1988a,b). There is little work to identify the level of protein required to induce laminitis. Some work suggests that excessive protein ingestion produces an allergic-histaminotic reaction, protein produces toxins and nitrogenous breakdown products that cause lameness (Vermunt 2000).

Correct fibre quality was also reported to reduce clinical lameness and this should be of a coarse nature to promote the correct micro-flora and action of the rumen and rumination (Livesey and Fleming 1984; Vermunt 2000).

Wet, fermented grass silage fed to youngstock is a risk factor pre- and post-calving (Logue *et al* 2000) as claw hardness is significantly reduced (Manson and Leaver 1989; Offer, Logue and Leach 2000) and poor locomotion and lesion development at 20 weeks was significantly higher in the silage fed heifers (Offer *et al* 2001). This may be attributed to the wetter slurry as a result of feeding the wet silage diet which was also deemed responsible for an increased level of heel erosion (Leach *et al* 2000). Claw horn disruption is also significantly worse in cubicle housed cows when fed a wet diet (Webster 2000).

A toxic factor in barley has been suggested (Maclean 1971), which involves the metabolism of histidine into histamine, or an allergic reaction to barley would create histamine, although no relationship has been observed between claw lesions and feeding barley, distillers grain or sugar beet by others (Logue *et al* 1994).

Mycotoxins which may be a result of fungi-damaged feedstuffs and mycotoxosis can produce a haemorrhagic syndrome in cattle feet (Vermunt and Greenough 1994; Vermunt 2000) which may cause 'double sole' which can also be seen in maiden heifers at grass (Brizzi *et al* 1998).

Trace mineral supplementation was carried out by Nocek *et al* (2000) and feeding zinc, manganese, cobalt and copper complex significantly reduced the incidence of the most commonly observed lesions; sole ulcer by 10.8%, white line separation by 28.9%,

sole haemorrhages by 13.4%, digital dermatitis by 33.1% and double soles by 56.5%. It also reduced incidents of dorsal ridges by approximately 2% but not significantly.

Lipid content of the claw can also be manipulated by the addition of fats to the diet of dairy cows. Offer *et al* (2000) found that the addition of fish oils to the diet can significantly alter lipid profiles in blood and claw horn. They suggested that changes in cell membrane function or inflammatory processes may have caused the differences observed in claw horn lipid profiles between lame and non-lame cows and fish oil dietary supplementation may reduce the level of lameness.

Biotin

In 1901 Wildiers initially developed 'bios', required for the growth of some yeasts, which resulted in the isolation of a pure form of biotin by Kogl in 1935. The structure was deduced by du Vigneaud and others in 1942 and the first synthesis was achieved by Harris *et al* in 1943 (Whitehead 1988). Identical to biotin is 'protective factor X' and 'vitamin H' (H = Haut meaning skin), also co-enzyme R (Whitehead 1988). There are four optically active forms of the biotin molecule although only one structure is biologically active (D - (+) - biotin).

Biotin is known to be necessary for normal reproduction and health (Roberts and Baggot 1982, Whitehead 1988). Biotin is a cofactor for enzymes in amino acid metabolism, cellular respiration, gluconeogenesis and lipogenesis. The biotin containing enzymes are, pyruvate carboxylase, propionyl CoA carboxylase, β-methylcrotonyl-CoA carboxylase (located in the mitochondria) and acetyl CoA carboxylase (located in the cytosol), and are important in carboxylation (Whitehead 1988; Roberts and Baggot;

Weiss and Zimmerly 2001). These enzymes are inactive until biotin is linked to lysine residue in the active site. This occurs via ATP dependent reaction catalyzed by the enzyme, biotin protein ligase. In effect, these are reactions where a transfer of carbon dioxide occurs from one compound to another (Roberts and Baggot 1982).

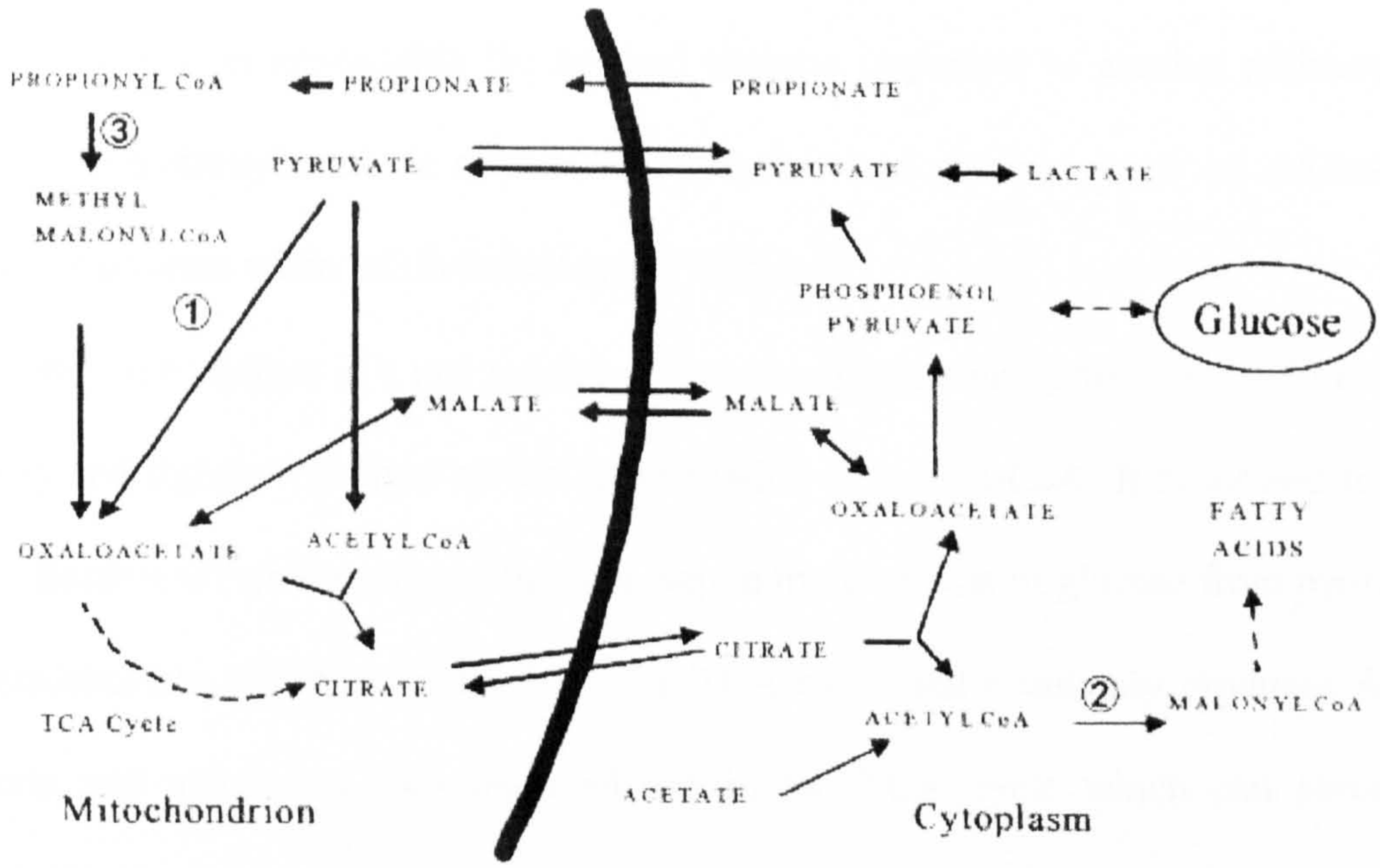


Figure 1.5: Gluconeogenic and lipogenic pathways that involve biotin. φ pyruvate carboxylase; κ acetyl-CoA carboxylase; λ propionyl-CoA carboxylase. β-methylcrotonyl-CoA carboxylase participates in the leucine catabolism pathway and is not shown (taken from Weiss and Zimmerly 2001).

The function of these enzymes (Figure 1.6):

- Acetyl-CoA carboxylase: catalyzes the ATP-dependent carboxylation of acetyl-CoA, which leads to the formation of malonyl-CoA, several units of this synthesise fatty acids and is recognised as the rate limiting step of lipogenesis. It is tightly controlled

but a key enzyme in a central metabolic pathway. This enzyme has a high priority for biotin and is probably not modulated by availability until a severe deficiency occurs.

- β-methylcrotonyl-CoA carboxylase: catalyses the conversion of β-methylocrotonyl-CoA to β-methylglutaconyl-CoA and leucine is eventually catabolised into acetyl-CoA and acetoacetic acid as long as biotin is available. This enzyme has a low priority for biotin and if it is unavailable the original enzyme is pushed to another pathway to produce 3-hydroxyisovaleric acid and 3-methylcrotonyl glycine which are evident in urinary excretion when biotin deficiency is marginal.
- Pyruvate carboxylase: is a key regulatory enzyme of gluconeogenesis in the liver and kidney and tightly regulated by the concentration of Acetyl-CoA. It is located in the mitochondria where it catalyzes the first step in the synthesis of glucose from pyruvate to oxaloacetate which is oxidised via the TCA cycle and eventually produces ATP. Citrate and malate are two intermediates in the TCA cycle which can serve as substrates for fatty acid synthesis and gluconeogenesis. Pyruvate is an intermediate in the conversion of amino acids (alanine, cysteine, glycine and serine) and lactate into glucose. This enzyme is reported to have a high priority for biotin and mild deficiency may not have a significant effect on enzyme activity.
- Proprionyl-CoA carboxylase: is a key enzyme in the catabolic pathway of odd-chain fatty acids, isoleucine, threonine, methionine and valine. The enzyme catalyzes the conversion of propionyl-CoA into methylmalonyl-CoA, which in turn enters the tricarboxylic acid cycle via succinyl-CoA then to oxaloacetate and eventually gluconeogenesis. This enzyme does not have as high a priority in other species as it does in cows because cows rely on being able to produce high levels of glucose.

Availability can limit milk production and proprionate is the major glucogenic precursor and makes the conversion from proprionyl-CoA into methyl malonyl-CoA very important. Therefore biotin is necessary and depleted levels may have detrimental effects.
(Dakshinamurti and Chauhan 1988; Sarasin 1994; Weiss and Zimmerly 2001).

In summary these enzymes are important in:

- Gluconeogenesis, fatty acid synthesis, amino acid catabolism in mammals and in proprionic acid production in bacteria.
- Formation of adenine and guanine (building block in DNA and RNA).
- Conversion of ornithine to citrulline in the urea cycle.
- Stimulation of cyclic GMP, and DNA transcription to RNA in cell culture.

(Sarasin 1994; Seymour 1998).

Biotin synthesis

Ruminant bacteria are able to synthesize biotin within the small intestine where chief absorption occurs (approximately 2mg per day more production than the rumen) (Miller *et al* 1986a,b; Zinn *et al* 1987; Frigg *et al* 1993) and in the rumen and large intestine (Weiss and Zimmerly 2001). Synthesis occurs hydrolytically with acid digestion. Many of the predominant cellulolytic bacteria of the rumen need biotin for growth (*Bacteroides succinogenes, Bbutyrivibro fibrisolvens, Ruminococcus albus and Ruminococcus flavefaciens*). High forage or digested organic matter promotes the natural biotin synthesis and excretion from the gut (Steinberg *et al* 1995; Abel and Da Costa

Gomez 1997; Da Costa Gomez *et al* 1998). Although no effect of diet on biotin has been found by other workers (Miller *et al* 1986a,b).

Biotin from the rumen is not degraded as it passes through the ruminant, and biotin supplementation does not effect systemic metabolism of biotin (Zinn *et al* 1987; Frigg *et al* 1993). Synthesis approximately ranges from 0-10mg per day in steer calves (Zinn *et al* 1987; Miller *et al* 1986b). From the findings of Zinn *et al* (1987), Weiss and Zimmerly (2001) estimated that a typical lactating dairy cow, based on an assumed intake of 20kg dry matter and digestibility of 65%, would produce approximately 10mg of biotin a day. Klunter *et al* (1993), however, estimated that 4 mg per day would be synthesised by lactating dairy cows. No biotin was found to be synthesised by the cows in the study by Frigg *et al* (1994). Actual levels of biotin synthesised by cows and the influence of factors on synthesis still remains uncertain (Weiss and Zimmerly 2001).

An increase has been observed between biotin intake with feed and excretion in faeces depending on diet (Miller *et al* 1986a,b; Steinberg *et al* 1995; Abel and Da Costa Gomez 1997). High concentrate rations suppress biotin synthesis or increase biotin degradation in the rumen (Abel and Da Costa Gomez 1997), however, many studies do not take into account biotin absorption by the gut for its own use. Natively bound biotin in many feed components can be lost and unavailable biotin has been located within traditional feed rations and both result in a potentially low natural available biotin intake per unit of production (Whitehead 1988; Abel and Da Costa Gomez 1997).

Biotin in other species

Details of biotin deficiency and the effects of supplementation in sheep and goats, pigs and horses are summarised in the following sections.

Biotin supplementation and deficiency in sheep and goats

Deficiency has not been observed, but can be induced in young animals, although deficiency-like signs such as soft horn, horn lesions and skin dermatitis are still observed (Whitehead 1988).

In sheep there is some evidence to suggest that fasting ewes close to lambing have insufficient biotin for gluconeogenesis and following biotin supplementation an increased rate of glucose was observed to be available to the sheep (Whitehead 1988 cited Kempton *et al* 1978).

Biotin also significantly effects follicle viability of wool ($P<0.05$) and the level of ATP contained at 0.5 or 1mg biotin per litre in culture medium. Increasing biotin levels also significantly increases follicle uptake of [U-^{14}C]-leucine and even though it was not significant, follicle DNA concentration has been seen to increase with increasing biotin levels (Scaife *et al* 1997).

Biotin supplementation has been seen to improve deficiency signs in Angora and Cashmere goat kids (hair loss around the ear, mouth and nose and decreased growth). Symptoms disappeared 2 to 3 weeks after the introduction of creep feed which corresponded with the development of the functional rumen. Biotin supplemented kids also had a significantly higher dry matter intake and higher horn length and circumference at the base, midpoint and tip (Scaife *et al* 1997).

Data on plasma biotin levels show that after supplementation of 100µg biotin per kg milk replacer, Cashmere kids had levels in the range of 150-1500pg biotin per ml plasma, deficient diets yielded 50-75pg biotin per ml plasma (Scaife *et al* 1997).

Biotin supplementation and deficiency in pigs

Monogastrics do not synthesise biotin and therefore need an external source. Deficiency causes decreased growth, decreased feed conversion efficiency, reduced reproductive performance, changes and lesions in skin (particularly the tongue and around the mouth and eyes) and reduced hoof horn and body tissue compositional quality (increased proportion of unsaturated fatty acids) (Whitehead 1988).

Feet often become crumbly and the horn loses strength and wear resistance (Webb *et al* 1984). Necrosis of the peripheral stratum corneum and disappearance of the ATP-ases near the basal layer have been observed. A higher incidence of cracks also develop in the claw in the coronet and distal wall and specifically at the tissue junctions for example wall to white line which may result in necrosis. Other factors such as rough flooring are required to induce severe lesions and resultant lameness (Whitehead 1988; Fritsche *et al* 1991).

Biotin synthesis in large amounts has been found from fermentation in the large intestine of pigs, particularly after a starch infusion (Mosenthin *et al* 1990), although bioavailability is limited, 1.7 to 17% of the metabolic requirements (Scholtissek *et al* 1990). Plasma or urine biotin concentration may not alter as a result however (Mosenthin *et al* 1990). The major site for absorption of biotin is the small intestine (Kopinski *et al* 1989; Mosenthin *et al* 1990).

Plasma and milk biotin levels are significantly higher in pigs supplemented with biotin than unsupplemented pigs (Bryant *et al* 1985b; Kornegay 1986).

Skin lesions are not always biotin responsive however. Some field trials have seen an improvement after supplementation of biotin, in some cases only gradually. In cases where a deeper infection are observed such as Greasy Pig Disease, some have reported a resistance to infection, which was seen when a large supplementation of biotin was applied (Whitehead 1988).

Many studies have recorded a positive effect of biotin in reducing foot lesions in pigs (Brooks, Smith and Irwin 1977; Money and Laughton 1981; Penny *et al* 1980; Bryant *et al* 1985a,c; Whitehead 1988), which have also had a previously depleted reproductive performance and improved the rate of return to oestrus and increased litter sizes (Brooks Smith and Irwin 1977; Simmins and Brooks 1983; Bryant *et al* 1985b). Litter size effects and reduced days to conception have been disputed (Greer *et al* 1991).

An improvement in claw compressive strength and hardness of mid abaxial side wall horn has also been observed as a result of 1mg d-biotin per kg diet (Webb, Penny and Johnston 1984), reduced foot 'splay' and lesions from pork weight (Brooks and Simmons 1980; Penny *et al* 1980; Johnston and Penny 1989), a greater number of tubules of a more cohesive and better defined structure and laminar horn structure more tightly packed and cohesive (Kempson *et al* 1989; Johnston 1990).

Fritsche (1990) *in vitro* cellular investigations has proposed that biotin inhibits the premature decay of horn cells, perhaps owing to an influence on the keratinocyte cell membrane or on the membrane coating material, therefore it directly stimulates an increase in cytokeratins resulting in the stimulation of epidermal cell differentiation.

Sarasin (1994) discovered an effect of biotin on the growth of isolated outer root sheet cells (ORS), *in vitro* model. Biochemically, biotin treatment resulted in significant cell proliferation without effecting differentiation, increased nucleation of the cell layers, an increased DNA replication and an influence in the expression of specific keratins.

Biotin supplementation and deficiency in horses

Dietary inclusion was introduced to horses as a result of the success observed in pigs (Linden 1992). The daily biotin requirement for the horse has been estimated at 1-2mg and this was considered to be provided by synthesis in the caecum and colon (Buffa *et al* 1992). Even though deficiency has not been observed and the horse is thought to require dietary biotin only in stressful conditions, some successful supplementation studies have been carried out on horses with weak hoof horn (Kempson 1990; Comben *et al* 1984; Linden 1992; Josseck *et al* 1995).

It is not fully known whether these problems reflect a deficiency or are abnormalities in the horn, potentially heritable (Zenker *et al* 1995), that are biotin responsive (Whitehead 1988). Abnormalities include cracks and fissures, low heels and concave wall.

Supplementation of biotin significantly increases horse plasma biotin levels more than 3-fold (Josseck *et al* 1995).

Significant improvements have been observed in white line (particularly terminal horn) and horn quality in horses supplemented with biotin compared to the unsupplemented (Comben *et al* 1984; Schulze and Scherf 1989) after 9 months (Josseck *et al* 1995). Small improvements have also been noted in quality histologically and tensile

strength in the biotin supplemented horses but not until after 19 months of supplementation (Zenker *et al* 1995). However, Kempson (1987) found limited improvement in response to biotin supplementation in individual horses with brittle feet.

Hardness and hoof conformation (various levels of reduced concavity, thicker soles and stronger, deeper heels) have also been improved by biotin supplementation (six to nine months supplementation was recommended) (Comben *et al* 1984). Supplementation of biotin has also been reported to increase horn growth rate and hardness (more evident in the toe and quarters), although when the rate of growth of horn was considered, these changes had occurred prior to the start of supplementation (Buffa *et al* 1992).

Biotin deficiency in cattle

The occurrence of biotin deficiency in cattle is a relatively sparse subject since an inadequacy has not been identified in dependent pathways, even in times of high metabolic activity (Roberts and Baggot 1982; Whitehead 1988; Frigg *et al* 1993 and 1994). Deficiency has been reported in poultry and pigs and in one study in calves.

Paralysis was observed in the hind legs of calves but have not been repeated (Hurstel 1982 cited Wiese *et al* 1946). Deficiency like symptoms such as hair loss, soft and crumbling hooves, skin lesions, alopecia, depigmentation, scabby dermatitis, reduced liveweight gain and feed conversion and increased scouring have also been observed in calves which responded to biotin administration, except for weight gain and feed conversion (Zinn *et al* 1987). Some of these symptoms have also been observed in the

cow (Roberts and Baggott 1982; Zinn *et al* 1987; Budras *et al* 1997; Galbraith *et al* 1998).

The histological observations of calves' hoof horn during deficiency were that basophilic keratohyaline granules in the heel of the normal hoof were missing and no distinction existed between the keratinising and cornified cells. The horny layer contained basophilic spots consisting of keratohyaline granule remnants that are normally dissolved. Stabilising filaments of the cell centre and the cell membrane were effected, and proteins were lacking. Lipid components were extremely depleted, to the extent that the periople, which normally contains stacks of membranes formed from complex lipids, was not evident (Budras *et al* 1997).

Deficiency may be induced by the addition of avidin, a glycoprotein that binds biotin, to the feed of young ruminants and additional dietary components, including drugs, can alter ruminal synthesis and/or cause destruction (Zinn *et al* 1987).

Biotin supplementation in cattle

Absolute metabolic requirements of biotin, however, still remain largely unknown and have warranted the need for further study (Zinn *et al* 1987). Biotin plays a significant role in gluconeogenesis, which is in high demand at times of high metabolic activity such as calving and early lactation, and protein synthesis and the rate of sceloprotein (keratin) production and deposition (Roberts and Baggot 1982).

Supplementation with biotin has improved fertility of Friesian dairy cows by an average of 1.93 services per conception (10 mg per cow per day protected biotin) and reduced disease incidence (udder and genital problems and claw lameness) by 60 to 70%.

It was also said to increase glucose, methionine, leucine, glutamic acid and marginally acetate, phenylalanine, glycine and histidine which are factors that have been indicated as positive for metabolic health (Bonomi *et al* 1996).

It may be that endogenous levels are insufficient in some animals where mild deficiency-like symptoms are observed in the form of poor horn quality. Therefore, even though absolute deficiency is not observed in cattle, the addition of biotin through dietary supplementation may assist in increasing the quality of the claw horn and reduce the incidence of lameness (Fritsche *et al* 1991). A similar process has been observed in studies in other vitamins such as Vitamin E (Batra *et al* 1992; Erskine *et al* 1997).

Supplementation of biotin orally significantly increases the intestinal output and utilisation of biotin (Zinn *et al* 1987; Frigg *et al* 1993). Several studies show that regular biotin supplementation is evident as a sustained increase in biotin concentration within the individual. This may be monitored in plasma, milk (a more reliable measure), urine and faeces (Steinberg *et al* 1995). After a single oral administration of 20 mg biotin a mean recovery of 97% was found; 10% in milk, 17% in urine and 70% in faeces. Intravenous administration of 10mg biotin still results in a mean recovery of 97%; 36% milk 61% urine and nothing in faeces (Steinberg *et al* 1995).

Biotin levels in plasma

Sarasin (1994) shows that the average levels of plasma biotin observed in cows is 1279 $\pm$ 76pg per ml, similar levels are observed in the rabbit and higher levels are observed in pigs (1345 $\pm$ 30pg per ml) and poultry (3722 $\pm$ 255pg per ml).

A significant linear relationship has been identified between biotin supplementation doses of 20 to 80mg per cow per day and an increase in plasma and milk levels (Frigg *et al* 1993, 1994; Klunter *et al* 1993; Distl and Schmid 1994; Campbell *et al* 1996; Hotchstetter 1996; Koller *et al* 1998; Midla *et al* 1998; Fitzgerald *et al* 2000). Below this level of supplementation only a small relationship has been found (Klunter *et al* 1993; Frigg *et al* 1994). Roberts and Baggott (1982) found conversely that plasma biotin levels dropped sharply after 8 weeks of biotin supplementation until 4 months, although their study was small, Voigt *et al* (2000) did not observe a difference between unsupplemented and 10mg biotin supplemented cows, although significant differences were observed in the milk samples. Hochstetter *et al* (1996) suggested that even with high biotin uptake low plasma levels may be possible.

All plasma biotin values, in supplemented and unsupplemented alike, may vary as a result of sampling time, individual cows and daily fluctuation (Frigg *et al* 1993) in relation to biotin supplementation. The following describes the pattern of circulating biotin following supplementation and it's influence: increased plasma biotin one hour after supplementation until four hours after the second supplementation and initial values are dependent on the level of supplementation (Klunter *et al* 1993). Or changes in general management, stage of reproduction, feeding, intervals of sampling are some of the other factors that have been reported to affect plasma biotin concentration (Whitehead 1988; Frigg *et al* 1993; Klunter *et al* 1993; Midla *et al* 1998).

Work carried out by Frigg *et al* (1993) compared heifers, treatment (20 mg biotin daily) and control groups which showed that plasma biotin levels rose from 300-800 ng/L to 3000-8000 ng/L with supplementation and there were no significant changes in

concentration associated with increasing time. Others have also found significant plasma biotin differences in supplemented and unsupplemented heifers, cows and steers (Frigg *et al* 1994; Koller *et al* 1998).

Biotin plasma levels are also different in lactating and non-lactating animals. Steinberg *et al* (1995) identified that following six months supplementation of biotin at 20mg per cow per day, lactating cow plasma biotin levels increased to 5.3nmol/L and 14.9 nmol/L in the non-lactating cows. A sharp increase of plasma biotin can be seen at calving only when cows are supplemented with biotin (Weiss and Zimmerly 2001). The level of plasma biotin in the supplemented cows 25 days after calving has also been seen to reduce, which may be related to increased dry matter intake (Midla *et al* 1998). Depleted plasma concentration of biotin in lactating cows has been observed in cows with a history of lameness, which may be because cows with a lameness history (Higuchi and Nagahata 2000) may also be higher yielding cows with a different diet (Roberts and Baggott 1982). An inverse correlation between plasma biotin levels and sole horn moisture content has been observed and also related to lame cows (Higuchi and Nagahata 2000).

Frigg *et al* (1994) highlighted the limited value of plasma biotin as it cannot identify the biotin content of tissues in general or specific tissues i.e. the epidermis, or the duration of any effect.

Biotin levels in milk

A linear relationship has also been found between biotin intake and concentration in milk over all dose ranges up to and over 80 mg per cow per day (Klunter *et al* 1993). The increased levels observed in milk biotin concentration represent 10% of the increased intake (Steinberg *et al* 1996;Weiss and Zimmerly 2001). Milk is more reliable for measuring biotin levels especially when intervals between sampling are long and only one sample per animal per day is taken (Klunter *et al* 1993; Steinberg *et al* 1996; Hochstetter *et al* 1996). Midla *et al* (1998) found that significant differences between biotin supplemented and unsupplemented cows only occurred after 293 days of supplementation and a large variability was observed between individual cows. They attributed their results to possible problems in milk handling and testing. When cows were not lactating a high percentage of the biotin intake is excreted in the urine (Steinberg *et al* 1996).

Colostrum levels of biotin have also been evaluated from cows receiving 20 mg biotin per day and five times the biotin levels seen in the milk of biotin supplemented cows has been identified. Unsupplemented cow colostrum was not found to have biotin levels any different from the levels seen in the milk of unsupplemented cows (Weiss and Zimmerly 2001).

Effects of biotin supplementation on milk production

There are very few studies that specifically observe the effects of biotin supplementation on milk production levels. The 305-day mature equivalent milk

production has been significantly higher for first lactation cows with biotin supplementation than the control cows (Bonomi *et al* 1996; Midla *et al* 1998). A significantly increased DHIA estimated milk production from the rolling average of 32.2kg per cow per day by 2.9kg per day or 878kg in 305 days has also been observed (Bergsten *et al* 1999, cited by Weiss and Zimmerly 2001).

A significant linear increase in milk yield of biotin supplemented cows in the first 100 days of lactation has been observed, not at the cost of increased tissue mobilisation (Weiss and Zimmerly 2001). However, Voigt *et al* (2000) found that milk yield in individual dairy cows increased significantly following the initiation of biotin supplementation, but unsupplemented animals did not have significantly lower biotin milk concentration compared to the supplemented cows, although few animals were used in this study. Steinberg *et al* (1995) identified a weak correlation between milk yield and milk biotin concentration.

It has been postulated that an increased milk yield equals an increased production of Propionyl-CoA carboxylases possibly increasing gluconeogenesis, and even though no increased biotin concentration is present in plasma it is thought that it may be because the mammary gland extracts the extra production for its own use, therefore the plasma samples appear to stay the same (Weiss and Zimmerly 2001 cited Zimmerly and Weiss 2000).

Other workers have not found a significant difference between supplement in milk yield (Fitzgerald *et al* 2000) but they have in other milk quality values such as a significantly increased protein content (Bonomi *et al* 1996), significantly reduced fat percentage and somatic cell count (SCC) (Fitzgerald *et al* 2000) in the milk of biotin

supplemented cows. Although Coulon *et al* (1998) found an increased SCC, which they associated with stress from walking with foot problems and Bonomi *et al* (1996) found a significantly increased fat concentration as a result of biotin supplementation.

Biotin bioavailability

Overall from previous studies, bioavailability of supplemented biotin has been estimated to be 40% and 56% in lactating and non-lactating cows, respectively from intraruminal dosing (Steinberg *et al* 1994; Steinberg *et al* 1995; Steinberg *et al* 1996). Frigg *et al* (1993) found a bioavailability overall at an average 48% in heifers (20 mg biotin daily, orally and intravenous) with a range from 40% in the heifers receiving biotin supplementation 26 days before the study to 55% found in heifers that did not receive biotin supplementation. Steinberg *et al* (1995) found that heifers had about 20% more bioavailable biotin than lactating cows. However, animals in lactation, as previously discussed, have rapid carbohydrate metabolism and fat synthesis which are demands made on the biotin-dependent pathways, therefore lower levels of biotin concentration may be expected in these animals due to a potentially increased use (Steinberg *et al* 1994; Steinberg *et al* 1995).

As a result of the studies to date, recommendations have been made for a daily intake of 20mg per day of biotin supplement for dairy cows to make significant changes in hoof horn.

Biotin and the bovine claw

Along with biotin, calcium and zinc are activators for enzymes that are essential for normal horn production and good quality horn (Budras *et al* 1998a). Biotin and zinc have in particular been reported to influence differentiation of epidermal keratinocytes (Whitehead 1988; Comben *et al* 1984; Fritsche *et al* 1991; Buffa *et al* 1992).

The maximum potential effect of supplementation with biotin is not expected until after approximately 15 months of supplementation because of the slow growth and renewal phase of the hoof. The full 15 month period is required for coronary horn to be fully replaced and the sole and heel horn require a 3-4 month period, as previously outlined. Prevention of growth of poor quality horn through nutrition and dietary supplementation with biotin, by influencing the growth quality in production rather than later modifying horn, is proposed to be a more advantageous (Mulling 2000).

Cell culture provided evidence that biotin in culture medium affected the differentiation of epidermal cells. It caused an increase in cytokeratins *in vitro* which would be indicative of terminal differentiation *in vivo*, without altering cytokeratin expression (Fritsche *et al* 1991). Even though this was *in vitro* it may explain the improvements in horn quality or structure observed *in vivo* (Schmid and Geyer 1994).

Condition of the hind claws only in biotin supplemented cows were examined in samples extracted from the abaxial end of the white line and the heel. Considerable improvements were seen in nine months and this was found to be as a result of inter and intracellular changes in the horn cells. Biotin influenced the amount of translatable mRNA of certain cytokeratins (Fritsche *et al* 1991), and changed the structure and composition of the intercellular cementing substance, which in turn was a result of biotin

mobilisation on the energy and lipid metabolism of the differentiating keratinocyte and therefore more localised availability to the producing cells (Fritsche *et al* 1991). Light and transmission electron microscopy also identified the presence, more clearly, of keratin filaments and their proteins and this was attributed to the changes in intermolecular linkages (Hotchstetter 1998). Similar to the findings of Johnston (1990) and Kempson *et al* (1989) in pigs.

It has been proposed that improvements in hoof horn condition are due to the rate of horn growth, however, an increase in growth rate has not been seen in other trials carried out on cattle (Schmid and Geyer 19+94; Josseck *et al* 1995).

An improved structure of and a return of intercellular cementing substance, previously largely missing, has been found after 12 months supplementation of biotin (Budras *et al* 1997). Lipids make up a large part of the intercellular cementing substance and biotin is essential for long chain fatty acid synthesis to make it more cohesive and able to control the aqueous and polar balance in the hoof (Budras *et al* 1997; Mulling 2000)

Other studies have observed significant horn quality improvements as a result of biotin supplementation in new horn developed following a lesion. The average complete coverage of horn was reported to take 15.9 days (Koller *et al* 1998). An improved rate of healing in the first few months of a severe lesion has also been reported, but no subsequent improvement in horn quality in the studies of Hunkeler *et al* (1996) and Lischer *et al* (1996). Koller *et al* (1998) however, did not find an increased rate of healing in sole ulcers. Hunkeler *et al* (1996) also found that severity of the lesion, previous lameness history and duration of lameness were correlated with the healing rate

Fitzgerald *et al* (2000) found that locomotion score was significantly correlated with the number of days of rain and that biotin supplemented cows had significantly lower lameness rates in the wet summer period than the unsupplemented herds. The definitive time was one year after the start of supplementation. No difference was observed in the dry period and their study was a between farm study, therefore other variables may have influenced the levels of lesions and lameness observed.

Like many other studies Fitgerald *et al* (2000) also found the lateral hind claw was affected more by lameness than the medial on the hind feet of cows. A significant reduction was found in the biotin supplemented compared to the unsupplemented cows of moderate to severe locomotion scores of the lateral hind claws ($P<0.05$). Hind feet had different damage scores; the right hind overall had a higher damage score than the left foot and biotin supplementation was significant in the left hind with lower scores.

Changes in claw conformation have also been observed and reported to be associated with increased plasma biotin levels. Biotin supplemented cows were observed to have a steeper angle of the dorsal border, and increased length and height of heel and the diagonal and ground surface increased accordingly in the hind foot (Distl and Schmid 1994).

Therefore, biotin supplementation has been reported to reduce lameness incidence (Cooke and Brumby 1982; Voigt *et al* 2000) and specific lesions which are discussed in more detail in the following section.

Biotin effect on specific lesions

From external efficacy studies summary conclusions with regard to biotin effects on bovine claw lesions have been made:

Campbell *et al* (1996) found that biotin supplementation in Hereford beef cattle, with a history of severe sandcrack lesions (37% prevalence in animals over 3 years of age) resulted in significantly increased average serum biotin concentration and significantly decreased prevalence of sandcracks, 14.3% and 29.4% respectively. Animals supplemented with biotin were estimated to be 2.5 times less likely to have vertical fissures.

In a field study of first lactation Holstein cows, biotin supplementation initiated after calving significantly reduced the incidence of white line separation in medial and lateral claws of the right hind limb and the lateral claw of the left forelimb (the only limbs examined) at 108 days after calving. A significant difference was not observed at 293 days post calving for white line separation by supplement (Midla *et al* 1998). A significant difference in sole haemorrhage score in the fore feet was also seen at 25 days post calving. Unsupplemented cows had significantly deeper fore claw heels than the supplemented cows. The depth of the claws in the fore feet, according to claw were observed to have different heel depths at different examination times, but overall differences were approximately 25% in the medial and 50% in the lateral claw (Midla *et al* 1998).

Distl and Schmid (1994) concluded that after biotin supplementation a reduction in the incidence and severity of interdigital dermatitis (after 2 months), sole ulceration and sole bruising (after 4 months) around the sole but not the white line, was found. Sole

ulcers and double soles, although rarely seen, were found more commonly in the unsupplemented cows. Heel erosion was found during the trial in both supplementations but severity had declined in the biotin supplemented animals at the second assessment at 16 weeks. Midla *et al* (1998) suggested that trauma was a significant factor involved in the prevalence of heel horn erosion and that the effect of biotin supplementation improving horn quality may not overcome inflicted insults.

Lower numbers of sole ulcers and heel horn erosions were observed in biotin supplemented cows by Voigt *et al* (2000), although the difference was not significant despite 15 months of biotin supplementation, which was considered to be because 10 mg of biotin per cow per day was insufficient to see a significant difference.

To date, many findings within trials on biotin supplementation have suggested a significant effect of biotin on lameness and accompanying lesions. General work on biotin supplementation in bovines has also been approached on a fairly small scale. Work to date, and the ever increasing realisation of the deleterious effects of lameness on production, has necessitated larger scale research into biotin supplementation and its effects in the reduction of lameness and improved milk production.

Aims

The aim of the trial was to observe the incidence of all types of lameness over an 18 month period and the hypothesis was that supplementation with biotin reduced the incidence of lameness and specific lesions in dairy cows. The trial also aimed to determine if biotin supplementation influences milk yield, compositional quality and cell

count. A well designed within herd intervention study observing 900 cows was carried out on 5 commercial farms in the UK.

CHAPTER TWO

MATERIALS AND METHODS

Selection, location and study design

Farm selection

Five farms were convenience selected (Martin *et al* 1987) on the following characteristics: routine herd health visits, had >90 Friesian/Holstein dairy cows, used the same veterinary practice to treat lame cows, and farmers expressed a genuine interest in the study for the entire 18 month period. The number of cow years required for the study was estimated using Epi info version 6.04 (Dean *et al* 1991). The sample size was selected to detect a relative risk of 1.7 with 80% power and 95% confidence, assuming an incidence of 42% to achieve a reduction in lameness of 20% with an exposure of 50% (1/2 supplemented with biotin). It was estimated that a total of 752 cow years split equally between supplementation with orally administered biotin and unsupplemented cows were required. Cows and heifers were selected from each of the five farms. All animals included were free from lameness, other diseases and any long term problems at the start of the trial, and represented a typical cross-section of age, parity and production.

Method of ID - Freeze brand identification on the hind-quarters were predominantly used to identify individual animals and records were scrutinized for recycled cow numbers. Ear tag identification were used for some heifers until they were allocated a freeze brand number.

Cows were stratified by the predicted calving date or closest date of last calving and allocated to receive biotin supplementation or not within these strata using random number tables. Heifers were assigned to either receive biotin supplementation or not by random allocation of the first heifer, by the toss of a coin, and then alternately allocating the rest of the heifers to either receive biotin supplementation or not. Animals entering the herd during the progression of the trial were randomly allocated to be either biotin supplemented or unsupplemented by tossing a coin.

The cows receiving supplementation were identified with a leg band (velcro material) placed around each lower hind leg above the fetlock. Farmers identified missing, old and damaged bands and replaced them. The researcher inspected the leg bands once a month to confirm that bands were placed on the correct cows and two bands were present on all supplemented cows (Plate 2.1).

Herd data collection

All data were collected and stored for analysis using Microsoft Access for Windows '95, (Version 7, 1989-1995, Microsoft Corp.). The following data were recorded:

Herd records - These included: cow identification (freeze brand), number of calves born in the trial, parity, lactation, last calving date, date of birth (not always available).

Plate 2.1 Identification of biotin supplemented cows with the use of leg bands

Ongoing records - Following the initial data, records were collected for each individual cow and its management throughout the 18 month period. This information included; biotin supplementation, calving date, drying off date, milk quality records, lameness occurrence, additional hoof trimming unassociated with lameness, foot-bathing, culls, deaths, animals sold, illness (mastitis etc.), date of grazing turnout and housing, type of housing, location of grazing turnout, method of moving animals (motor transport

or herd walking) and nutritional details, where available. Data were collected throughout the trial to monitor biotin administration and concentration on each farm (see later).

Between and within farm variables were considered to be a very important aspect of the trial. This was necessary for possible explanation in the final analysis, for example number of staff present, their education and job specification (part/full time, seasonal/casual), cow breed differences, production differences (e.g. milk yield etc.), farm location and size, housing system and herd management.

Farm location and staff information

All farms were located in Gloucestershire, UK. Farm 3 and 5 were located near the river Severn and Farms 1, 2 and 4 were located near Gloucester city.

The cows on Farm 1 were managed by a farm manager, a herdsman and a farm labourer. There was a regular turnover of students that assisted with the work on a daily basis. The herdsman milked the cows daily with the assistance of students. The relief milker was the farm labourer, who was also assisted by students. The herdsman was responsible for biotin supplementation of the lactating cows in the parlour and commonly assisted by students. In the event of a holiday, the relief milker supplemented the lactating cows, again with the assistance of students. Dry cow and heifer supplementation was carried out by the farm labourer with the assistance of students.

Farm 2 was managed by one herdsman who also milked the cows and administered biotin supplementation on a daily basis to lactating animals, dry cows and heifers. When the herdsman took a holiday he was replaced by a professional relief milker who also acted as a relief milker on one other trial farm (Farm 4) and continued

the supplementation process in the milking cows and fed the dry cow supplement feed as directed.

Farm 3 was managed by a herdsman and the farm owner. The herdsman carried out the daily feeding and milking and therefore all biotin supplementation and the unsupplemented feed ration in the case of dry cows and heifers. Relief milking was initially, for a short period (approximately 4 months), performed by an outside relief milker, then the owner performed any relief milking maintaining biotin supplementation in the parlour. A farm labourer administered dry cow and heifer feeding and biotin supplementation when the herdsman was on holiday.

Farm 4 was managed by one herdsman and the farm owner. The herdsman milked the cows and supplemented the cows with biotin in the milking parlour. The farm owner fed biotin supplemented and unsupplemented feed to the dry cows and heifers and prepared the feed in the milking cow house. The relief milker was the same person who covered holiday periods on Farm 2 and was very familiar with the biotin supplementation system in the parlour.

On Farm 5 the cows were managed by a family of three. One performed the majority of the milking and one other very occasionally milked the cows, both members supplemented biotin when they were milking. When the predominant milker was on holiday a relief milker was brought in who continued parlour biotin supplementation. All three family members were involved in feeding the dry cow and heifer biotin supplementation and unsupplemented ration.

Management/housing/grazing

All the milking cows were kept as one herd whether supplemented with biotin or not. They were housed during the winter and turned out for grazing through the summer.

The trial cows were at pasture at the beginning of the trial (June-September 1997) and were put out to grazing later than normal in 1998 as a result of wet weather (April/May). Farmers initially allowed access to housing at turn-out, or housed overnight with turn-out to pasture during the day.

Housing took place in both the first and second winters of the trial in late October. There was a period of approximately 1-2 weeks where milking cows on Farm 1 were housed during July 1998 because the fields were used for an equine event, but they returned to grazing afterwards. Grazing was very limited in the 2nd summer of 1998 due to wet weather earlier in the year and silage feeding started earlier than normal on all farms, in July/August.

Lactating animals - In the summer all cows were turned out onto pastures surrounding the buildings and walked into the parlour for milking on stone tracks and concrete. All farms had relatively short distances between the fields and the milking parlour (maximum of approximately 400 metres).

All winter housing was located next to the milking parlour and included concrete walk-ways with a central feed passage and cubicles for Farms 1, 2, and 4. Farm 5 had the feed area separate to the cubicles and an outside loafing area. Automatic scrapers were used on Farm 4. Farm 3 cows were loose housed and kept in straw bedded barns with a central feed passage. On four of the five farms fresh calving cows that were milked in the parlour were loose housed separately on straw the other farm (5) ran the cows with the

rest of the milking herd. All cows were milked in herringbone parlours after collection in a concrete yard.

On all farms nutrition of the lactating cows included mainly grazing and parlour concentrate in the summer months and the following rations in the winter: -

Farm 1 - In parlour dairy pellet concentrate and silage was fed in the feed passage at 30kg grass silage and 15kg maize silage per cow. High yielding cows received a different diet of 1kg barley, 2kg soya meal and 2kg caustic soda treated wheat. Low yielders: 30kg grass silage and 15kg maize silage.

Farm 2 - In and out of parlour concentrate (typically - 20-24% protein, 4.5% oil, 10.5% fibre, 8.0% ash, 0.6% magnesium). Concentrate was fed from feeders outside the parlour accessed by electronic collars which differentiated between the high and low yield diets. Higher yielders received better quality and higher protein feed. Grass and maize silage was fed and ranged from 50:50 to 30:70 respectively depending on the amount available. Brewers grain was fed (10kg /day) in the spring following the first winter of the trial.

Farm 3 - In parlour concentrate was fed to placate the cows whilst milking (typically - 19% protein, 3.5% oil, 11% fibre, 8.0% ash, 0.56% magnesium, 13.8% moisture). Concentrate was also fed in the feed passage in the form of a complete diet, straights - brewers grain, wheat, soya, maize gluten. High and mid yielders were fed the same diet and low yielders had less straights by 2/3. Silage was fed at 40% grass and 60% maize.

Farm 4 - In parlour concentrate was fed in the form of standard dairy pellets. A semi-complete diet was fed in the feed passage of the yard. The diet was mixed on site and incorporated wheat, soya, mix and varied according to the availability of the ingredients. Silage was fed at a rate of 25% grass and 75% maize. All lactating animals were fed identically.

Farm 5 - In parlour concentrate only, in pellet form (typically - 19% protein, 5% oil, 9% fibre, 8.5% ash, 0.5% magnesium, moisture 14%). Silage was fed at a rate of 50:50 grass and maize.

The dry cows and heifers - were kept in different groups according to whether they were supplemented with biotin or not. These groups were maintained in as identical conditions as possible. All heifers and dry cows on all of the farms were kept on separate fields during the summer and housed in the winter period, on straw, again in separate accommodation.

Pasture for trial dry cows was located away from the main farm site for a short period during the trial for Farms 3 and 5. Farm 5 walked all of their dry cows to the pasture which was approximately 1 km from the main farm site and the animals returned on foot for calving. Farm 3 transported their cows by vehicle to and from the grazing site. Supplementation was maintained throughout this period, which was evident in the reconciliation of dry cow rolls used and plasma biotin levels observed.

Dry cows and heifers were fed grass in the summer months and the winter ration included:

Farm 1 - Haylage or hay. Cows separated approximately 3 weeks prior to calving and given 2kg caustic soda treated wheat, 1kg barley and *ad-libitum* straw.

Farm 2 - Grass silage (20-30kg each cow) or *ad-libitum* straw. Dry cow roll (16-18% protein) 1-2kg each cow. Wheat straw was given in the second winter. Cows were separated for calving and joined the milking herd shortly after.

Farm 3 - Grass silage *ad-libitum*. Standard dry cow rolls. No change in diet prior to calving and joined milking herd after calving.

Farm 4 - Grass silage *ad-libitum*. Standard dry cow rolls. Dry cows separated from the rest of the herd 2-3 weeks prior to calving and joined milking herd 1-2 weeks after calving.

Farm 5 - Hay and grass silage *ad-libitum*. Standard dry cow rolls. Dry cows joined the milking herd approximately two weeks before calving.

<u>Foot care</u>

Foot baths were located on four of the five farms. Farms 2 (zinc crystals) and 4 used foot baths every month. Farms 1 and 5 used a foot bath if there was a significant problem e.g. large number of digital dermatitis cases. Farm 3 did not use a foot bath.

Foot trimming was carried out by veterinarians on Farms 2, 3, 4 and 5. On Farm 1 foot trimming was carried out occasionally by the herdsman but mainly by the

veterinarian. Routine foot trimming was carried out by the vet on Farm 4 before the cows were 'dried off'.

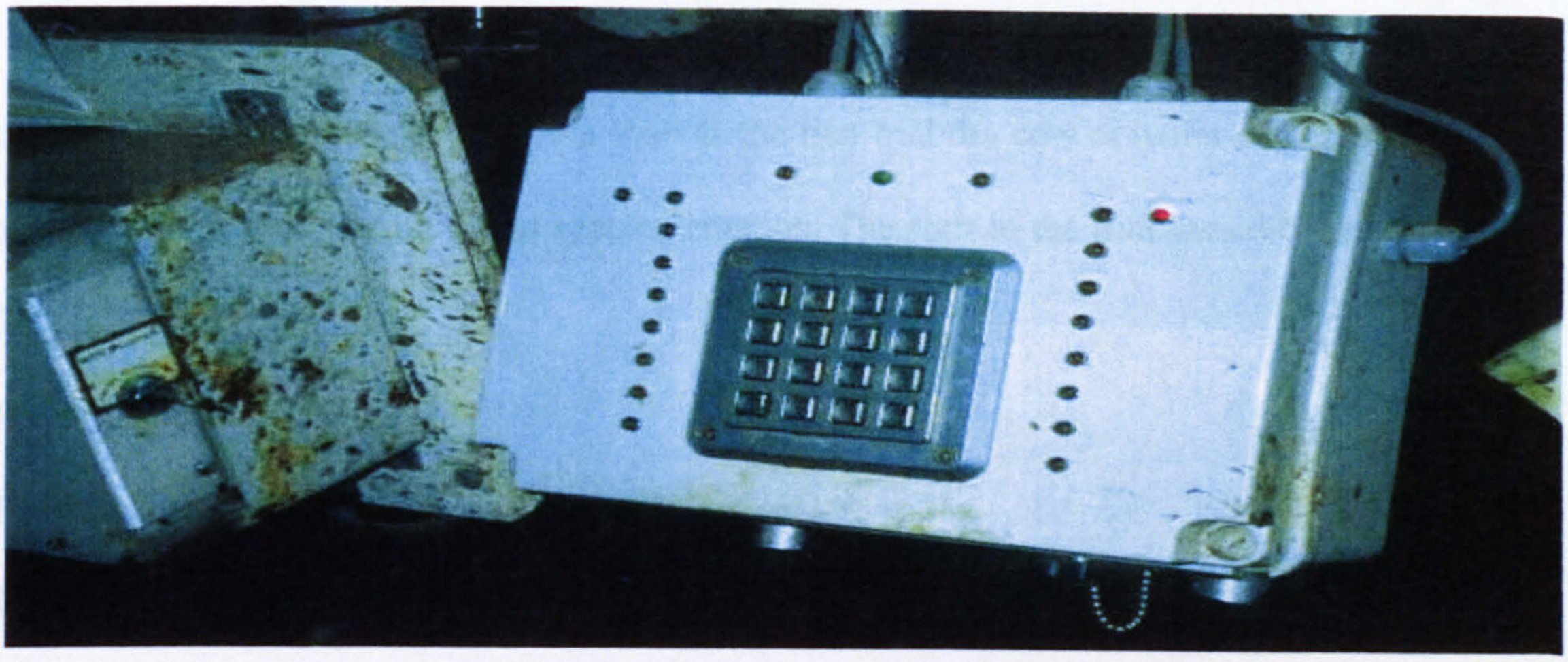
Biotin administration

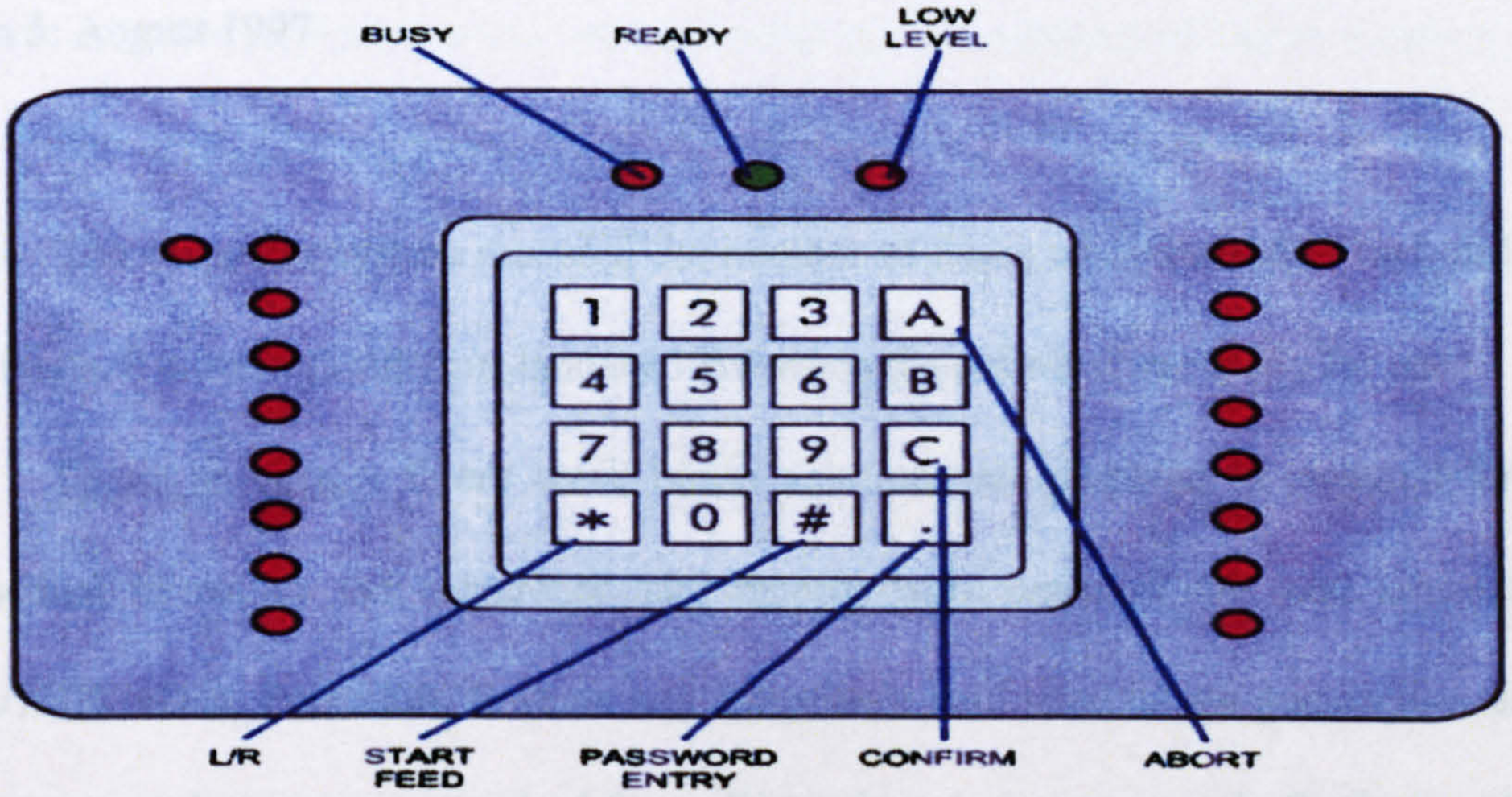
On each farm two calibrated header tanks were located above the milking parlour for the administration of biotin in solution to lactating animals (Plate 2.2). The upper tank had a clear measurement tube (litre increments) located on the outside which ran for the depth of the tank vertically and enabled easy solution level monitoring.

Both header tanks contained a heating element which kept the solution at 15-20^{0}C to avoid freezing. The lower tank contained a pump which kept the biotin solution circulating constantly around the pipe ring main which traveled the circumference of the milking parlour above the feed troughs. Above each feed trough a down-pipe descended from the pipe ring to a solanoid time release valve. The down pipe was lagged to protect it from freezing in the winter. Each release valve was individually programmed to the nearest 10th of a second to release 25ml of solution. This was achieved by entry into the computer system using security coding (known only by the researcher and system designer) and typing in solanoid valve opening times (seconds) for each dispensing point using the parlour keypad (Plate 2.3).

Plate 2.2: Solution storage tanks located above the milking parlour

Lactating dairy cows - A 25ml dose containing biotin at 0.04% (10mg) (2% ROVIMIX H2®) as aqueous solution was added to the compound feed of the supplemented animals twice a day at milking. The biotin in solution was dispensed into each individual feeder in the parlour by an electronic keypad (Plate 2.3).





Main Control Unit

Function of Keys:

1-9	Used to select/deselect the stall number you require.
0	Used to select/deselect point 10 (depending on site).
*	Used to select left or right hand side.
#	Will start the feed cycle and ask you to confirm this.
C	Confirm. Used to confirm the start of a feed cycle.
A	This key will abort a feed cycle if # was pressed accidentally
.&B	Used for password entry routines (DO NOT USE). Entering a number four times will abort if pressed accidentally.

Plate 2.3: Control panel located in the milking parlour for dispensing biotin supplementation to lactating cows

This system was operated by the herdsman who was trained in the use of the keypad or electronic control panel. As soon as the first trial dry cow or heifer calved, the milking herd started parlour biotin supplementation. The start of the trial varied between farms:

Farm 1: July 1997

Farm 2: June 1997

Farm 3: September 1997

Farm 4: September 1997

Farm 5: August 1997

The computer system recorded the number of doses administered at each milking and faults. Recording started in January 1998 when the software was ready for use.

Biotin solution - Every week biotin solution was prepared as required for the individual farms, by the researcher, and records were made of the total amounts of ROVIMIX H2®, Potassium Sorbate and Proprionic acid used in the preparation of the concentrate solutions on a standard form. Biotin in solution was made firstly in a 10 % solution and diluted to a 2% solution in the following process:

10% solution:-

100 grams of ROVIMIX H2® per litre of water

25 grams of Potassium Sorbate per litre of the ROVIMIX H2® and water solution

Dilution to 2%:-

Dilution factor of 5

1 litre of 10% solution required the addition of 4 litres of water to equal 5 litres of 2% biotin solution.

Post addition of proprionic acid at 0.1%, 1ml per litre of 2% solution.

Each container carried 22 litres of 2% solution.

The researcher took the containers of biotin solution to each of the farms to fill the header tanks for milking parlour supplementation. The amount of biotin solution (2% ROVIMIX H2®) added to the tank and amount already present were recorded and compared with the cows in milk, which were recorded on a standard form and compared each week to assess biotin use, an example can be seen in Appendix I.

In addition to this process, each individual dispenser was calibrated manually by the researcher each month to check that the computer system dispensed the required 25 ml biotin solution. Figures were recorded on the standard form (Appendix I). It was possible to use the dispensing system without adding data to the computer records by entering a code into the keypad before and after the checks and re-programming of the time release valves in the solanoids. However, the dispensing check data were recorded with the other dose data for the first 4 months until this option was available. It was necessary to recalibrate the 25ml dose twice in the trial, once on Farms 2 and 4 and also because of the problems encountered with a power surge and when cows damaged pipe work. Only the system designer and the researcher (VJH) were aware of any system codes and its functions, so changes and data downloading or checking could not be made by

anyone else. Data on the number of daily doses of biotin solution used were downloaded from the system to an external 'lap-top' computer.

Each month a 50ml sample of biotin solution was taken from the header tank before it was refilled to analyse the biotin concentration. This sample was stored at -20^{0}C before being shipped to the laboratory (F Hoffmann La-Roche Ltd., Basel, Switzerland) for biotin assay analysis. Data were stored using Microsoft Access for Windows '95, (Version 7, 1989-1995, Microsoft Corp.) and analysed using Minitab 10-5 (Minitab Inc.).

Although samples were collected every month, biotin analysis was carried out on quarter year samples. Data on the analysis of biotin content of the solution samples is shown in Table 2.1. The mean values for all farms were lower than the expected value (400 mg/kg) at which the solution was prepared. Differences in the observed and expected levels ranged from 11.37 - 23.22 % although no significant differences were observed between the expected and actual biotin concentration on each farm with *t*-test analysis (P 0.91). When the data were placed in date order (Figure 2.1), with five samples collected in each period, there was a difference in the concentration of biotin found in the solution between those sample times. Solution preparation followed a standard methodology with no deviation from the technique. New batches of Rovimix H2 were used very infrequently, possibly changes may have occurred at the parlour tank level and may relate to the findings of the microbial screenings.

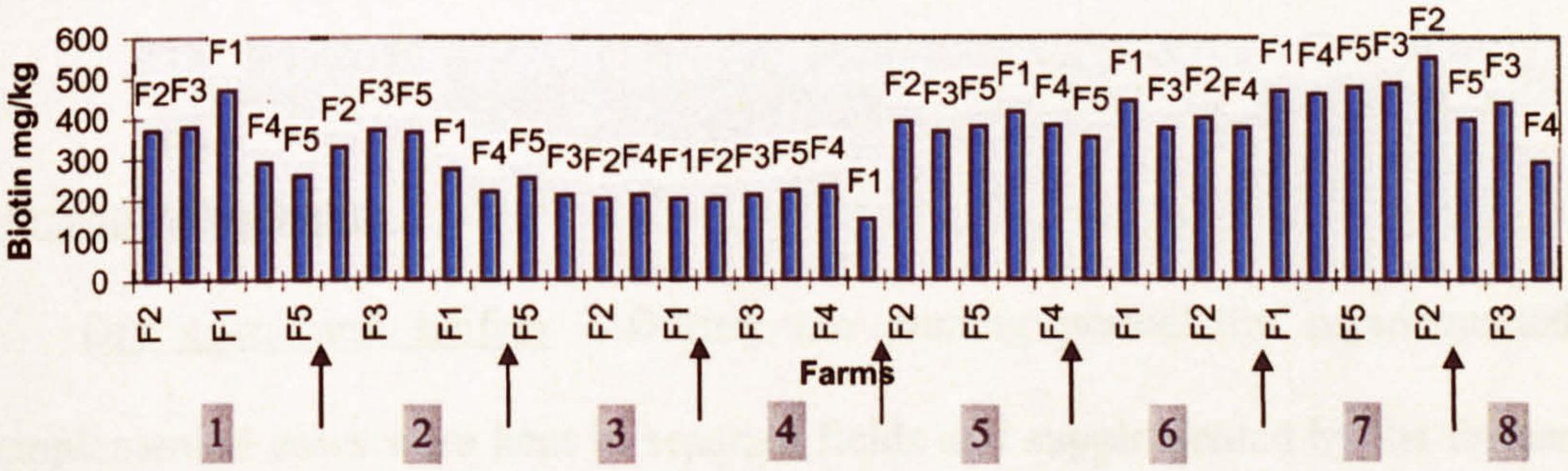
Table 2.1: Biotin levels observed in the parlour solution by farm

Farm No	N	Mean	St Deviation	Q1	Q3	Difference (mean to expected[1])	Difference (%)
1	7	346.3	135.8	200.0	470.0	53.7	13.42
2	7	349.1	122.4	200.0	402.0	50.9	12.72
3	8	354.5	98.1	249.5	421.3	45.5	11.37
4	8	307.1	91.1	221.7	380.0	92.9	23.22
5	8	337.4	87.7	251.7	391.2	62.6	15.65

Note: all figures mg /kg

[1]Expected value 400 mg/kg

Figure 2.1: Biotin in solution concentration by farm and sample time



F1-5 = Farms 1 to 5

↑ = Separation of solution sample periods: 1, October 1997; 2, December 1997; 3, February 1998; 4, April 1998; 5, July 1998; 6, October 1998; 7, December 1998; 8, February 1999.

Each quarter of a year a 50ml sample was taken for microbial screening. The sample was analysed immediately for microbes at Gloucester Laboratories (St Oswalds Road, Gloucester), and tested for :

Total viable counts at 37^0C for 48 hours on PCA

Coliform counts at 37^0C for 48 hours on VRBA

Yeast and mould count at 25^0C for 5 days on RBCA

These results were scrutinized by one veterinarian who advised of any pathological significance in the results. If required the tanks were emptied and cleaned using Trigene disinfectant, which did not leave any residue. This was necessary on Farms 2 and 3 once during the entire period of the trial, on the recommendation of veterinary advice because of elevated coliform counts.

Biotin supplementation

Dry cows and heifers - During the grazing period the supplemented and unsupplemented cows were kept in separate fields and supplemented by the farmer with 0.5 kg per cow per day of dry cow rolls (BOCM Pauls, Ipswich, UK). Each pellet or roll on average measured 4cm in length and 1cm diameter and contained identical ingredients with the exception that the supplemented rolls had 20 mg additional biotin in each 0.5kg and preservative (ingredient of the rolls are detailed in Appendix II). The feed was measured out into large buckets that were marked with a 0.5kg scale and calibrated by the researcher using weighed trial feed. This was chosen as the most effective method accepted by the farmers for daily use.

As feeding was not normally carried out in the summer months, standard galvanized feed troughs were provided on all farms. The aim at the start of the trial was to start supplementation of the dry cows and heifers approximately 3 months prior to the expected calving date of the first calving cow/heifer on each farm. Unfortunately a few of the initial animals were supplemented for a shorter period and 3 heifers calved earlier than expected on Farm 2, the first farm to start. Also 2 heifers from Farm 5 were supplemented for a shorter period than three months as they calved earlier than expected due to unknown service dates. Once the trial had started, supplementation continued through calving, lactation and the dry period until the trial finished. Any new animals entering the trial i.e. replacement heifers, were supplemented approximately 3 months prior to calving where possible.

Checking biotin concentration in dry cow rolls - The dry cow rolls were delivered to the farm by the feed company (BOCM Pauls) in batches of 3 tonnes of both diets and were distributed in 2 tonne batches (1 tonne of each diet) to 3 farms (Farms 1, 3 and 4). The researcher took bags of the two diets from these farms to the two remaining farms as required.

Representative samples were taken from each batch of dry cow rolls and frozen and stored at -18 to -20^{0}C before all were transported for biotin assay analysis (F Hoffmann La-Roche Ltd., Basel, Switzerland). All data were stored using Microsoft Access for Windows '95, (Version 7, 1989-1995, Microsoft Corp.) and analysed using Minitab 10-5 (Minitab Inc.). The mean for the analysis of the biotin supplemented feed was 27.2 $\pm$ 13.7 mg/kg. The unsupplemented feed mean was 5.7 $\pm$ 11.1 mg/kg, greater than the requested 0.3 mg/kg by 5.4 mg/kg.

Each week a reconciliation of the actual consumption of dry cow rolls, and records of the total number of dry cows, heifers present and movement of bags from one farm to another, where necessary, were carried out by the researcher. This information was recorded on a standard form (observed in Appendix I) and reconciled immediately. This highlighted inconsistent supplementation on Farm 1 with a lower use of both types of feed, specifically during periods where the herdsman was on holiday and supplementation was only at a rate of approximately 25% of required use. Inconsistencies in supplementation were brought to the attention of the person responsible for supplementation by the researcher and the attending veterinarian.

Even though the farmers had calibrated buckets to measure out the daily ration of dry cow rolls on each farm, the actual usage was complex to monitor because of spoilage and use varied by approximately $\pm$ 0.25 kg in daily total due to the size of the rolls and nature of measuring (examples of reconciliation shown in Appendix I).

Supplementation using dry cow rolls was readily accepted by the cows throughout the trial. There were doubts, expressed by the farmers or herdsmen, about the uptake of feed during the summer grazing period, but observations revealed that the great majority of the animals consumed the feed eagerly. Approximately 2 individual cows per herd were not eager to consume the feed at this time of year (Plate 2.4), these 2 individual cows were recorded but included in the final analysis.

Plate 2.4: The majority of the dry cow herd consuming the supplement feed during the summer

Monitoring cow biotin concentration and intake

In addition to the weekly records previously detailed, the researcher also attended morning milking every month to collect milk samples for biotin analysis, count the number of milking cows in the trial, check all cows were in their correct groups and that two leg bands were on the biotin supplemented cows. This method was successful in locating missing leg bands (one always remained) for replacement. A biotin supplemented cow with no leg bands was located on one occasion only. There was an

incident where a leg band was replaced too tightly and caused inflammation and lameness.

Milking cows - Milk samples were taken from animals in lactation to monitor the biotin concentration secreted. This is the most reliable method for monitoring biotin concentration within individual cows which previous studies have found and are detailed in the literature review. All samples were frozen after extraction at approximately -18^0 to -20^{0}C, before transportation for biotin microbiological assay analysis using *Lactobacillus plantarum* ATCC 8014 (F Hoffmann-La Roche Ltd). All data were recorded using Microsoft Access for Windows '95, (Version 7, 1989-1995, Microsoft Corp.) and analysed using Minitab 10-5 (Minitab Inc.).

A pre-trial 50ml bulk tank sample was taken in duplicate after morning milking, to record normal concentrations present in the herd overall. The concentration of biotin on the individual farms were:

Farm 1: 159.60 nmol/L

Farm 2: 94.15 nmol/L

Farm 3: 106.40 nmol/L

Farm 4: 122.80 nmol/L

Farm 5: 221.00 nmol/L

There were considerable differences observed between the trial farms for unknown reasons.

On Farm 4 only, milk samples were taken from a total of 20 individual animals before the start of supplementation, 10 that had been selected to receive biotin

supplementation and 10 that were going to be in the trial but unsupplemented. The data were analysed using *t*-tests, the intended biotin supplemented animals had a mean value of 87.18 ± 58.65 nmol/L biotin in milk and the unsupplemented had a mean of 112.97 ± 67.49 nmol/L. These values were not significantly different. When compared to the concentrations observed during the trial; Farm 4 pooled milk analysis (Table 2.2) and individual milk analysis (Table 2.3) increased by 207.92% (83.09 nmol/L to 333.33 nmol/L) and 211.60% (83.09 nmol/L to 337.75 nmol/L) respectively in the biotin supplemented cows. On the same farm a slight increase in the unsupplemented cows, 40.63% (112.97 nmol/L to 148.94 nmol/L) and 54.5% (112.97 nmol/L to 161.22 nmol/L) respectively was also found.

Each month every farm was visited at morning milking and 10 supplemented and 10 unsupplemented cows were randomly sampled. These samples were pooled by supplement into two 50ml samples for analysis and the remainder of each individual sample was stored at -18 to -20^{0}C. The first samples were collected from lactating animals a month after the first trial dry cow or heifer calved, entered the group and milking parlour supplementation started. The last sample was taken one month after the end of the trial.

The pooled milk sample data were analysed without pre or post trial samples by farm. A significant difference ($P< 0.001$) was observed between the biotin supplemented 453.22 nmol/L and unsupplemented cows 186.44 nmol/L. There was also a significant difference observed in milk biotin levels between supplemented and unsupplemented cows on all 5 farms (Table 2.2), although the standard deviations were wide. The data for biotin supplemented animals on Farm 1 was lower than the other farms and the

unsupplemented levels are comparable with the milk biotin observed in the bulk sample prior to the start of the trial. Therefore, data corresponds with the inaccurate supplementation procedures observed previously but still significantly higher than the unsupplemented cows (P 0.003). Despite the significantly different values of milk biotin observed between the supplemented and unsupplemented cows on Farm 3, the unsupplemented cows' milk biotin levels rose considerably when compared to the initial pre-trial bulk sample, from 106.40 nmol/L to 197.20 nmol/L, an increase of 96.61%.

Table 2.2: Pooled milk sample biotin levels by farm

Farm	Biotin	Mean (nmol/L)	St Dev	N	DF	P
1	Yes	283.68	157.29	16	1	0.003*
	No	151.44	50.59	16		
2	Yes	681.24	477.24	16	1	<0.001*
	No	189.80	79.03	16		
3	Yes	331.04	169.12	17	1	0.005*
	No	197.20	74.41	17		
4	Yes	333.33	145.91	16	1	<0.001*
	No	148.94	64.46	16		
5	Yes	633.23	292.57	17	1	<0.001*
	No	240.75	263.06	17		

P* significant

In addition to the 10 supplemented and 10 control samples taken every month, every third month five extra cows were randomly sampled from each group (5 supplemented and 5 unsupplemented). These samples were analysed individually. When

all farm data were pooled and analysed using *t*-tests, biotin supplemented animals (409.40 nmol/L ± 335.71) had significantly higher concentration (P <0.001) of milk biotin than the unsupplemented animals (172.64 nmol/L ± 164.54).

When the data were split by farm (Table 2.3) the problems previously highlighted in poor supplementation procedures on Farm 1 were obvious (P 0.10). Farm 3 was also highlighted in Table 2.3 (P 0.231) with a non significant difference observed between the levels of milk biotin in supplemented and unsupplemented animals. As previously discussed, the unsupplemented animals in Farm 3 had an elevated concentration of biotin in milk samples when compared to the pre-trial bulk samples. In the pooled samples (Table 2.2) the elevation was by 96.61% but in the individually analysed samples the difference, 106.4 nmol/L to 239.32 nmol/L, was an increase of 141.43%. It is suggested that these raised levels may have been a result of unsupplemented cows consuming some remnants of feed left in the troughs. This theory may be supported with the feeding regime adopted on Farm 3 where the majority of the concentrate ration was consumed outside the parlour.

The remaining 3 farms had a high significant difference in milk biotin concentration between the supplemented and unsupplemented animals (P<0.001) (Table 2.3). Farm 2 (436.35 nmol/L) and 5 (603.47 nmol/L) had the highest concentration of milk biotin observed in the biotin supplemented cows.

Table 2.3: Individual milk sample biotin levels by farm

Farm	Biotin	Mean (nmol/L)	St Dev	N	DF	P
1	Yes	242.71	228.67	30	1	0.100
	No	159.50	148.53	30		
2	Yes	536.06	436.35	30	1	<0.001*
	No	178.33	129.83	30		
3	Yes	343.40	384.99	30	1	0.231
	No	239.32	272.10	30		
4	Yes	337.75	142.07	30	1	<0.001*
	No	161.22	110.02	30		
5	Yes	603.47	268.70	25	1	<0.001*
	No	115.75	51.12	25		

P* Significant

The post trial milk samples were pooled samples and extracted one month after the final supplementation date on each farm in the same way as the samples collected every month. The data were again analysed using *t*-tests with pooled farm data comparing the previously biotin supplemented and unsupplemented animals. The mean concentration of milk biotin returned to the levels seen before the start of the trial, 92.5 nmol/L and 84.31 nmol/L respectively.

Plasma samples and biotin analysis - Blood samples were taken from dry cows and heifers approximately seven days before calving. These samples were taken by the trial veterinary surgeons to monitor biotin concentration in these animals.

HEIFERS - a single blood sample was collected from each heifer once by the visiting veterinary surgeon.

DRY COWS - 50% of the dry cows on each farm were blood sampled. In the event of several visits to achieve the required numbers, a few duplicates could have occurred. Revisits occurred on Farms 3 and 4 in the second dry season. On the other farms 50% of the herd were sampled at one visit.

The Samples were collected at least two days after the start of biotin supplementation as the half life of biotin is approximately estimated at 14 hours (Frigg *et al* 1994). Equal volumes of individual cow samples were pooled into supplemented and unsupplemented samples and divided into duplicate 10ml samples for analysis. For each group 5 random individual plasma samples, were also analysed (5 supplemented and 5 unsupplemented). All remaining samples were stored at -18 to -20^{0}C.

Data were stored in Microsoft Access for Windows '95, (Version 7, 1989-1995, Microsoft Corp.) and analysed using Minitab 10-5 (Minitab Inc.). The pooled sample analysis represents results taken from the entire 18 month period. However, individual plasma analysis represents only the second plasma collection period, when the second group of replacement heifers entered the trial, because during the first collection period insufficient quantities of blood were taken from some individuals to realise the required amount of serum for testing, and a number of samples were lost in transportation from storage to the analysis laboratory.

Samples taken from individual animals showed a significant difference of circulating biotin between the biotin supplemented animals and the unsupplemented animals (P 0.01). A significant difference was not observed between the heifers and cows (P0.76), or between the five farms (P0.64) involved in the trial.

The data were analysed further to consider interactions between supplementation, heifers or cows and farm overall (Table 2.4). The results produced a similar result. Supplementation remained the only significant variable (P 0.01) in the plasma samples taken from individual animals.

Table 2.4: Individual cow/heifer plasma biotin data ANOVA general linear model

Variable	Adj SS	F	P	DF
Supplementation	1566.7	7.21	0.01*	1
Heifer or Cow	132.5	0.61	0.44	1
Farm No	692.1	0.80	0.53	4

(nmol/L) P* Significant

Pooled samples of blood were analysed using 2 sample *t*-tests and no significance was observed when i.e. supplemented versus unsupplemented, cow versus heifer and the five different farms were analysed. When the data were further analysed, using the ANOVA general linear model (Table 2.5), figures for cow or heifer were significant (P 0.04). Supplementation was not significant in the analysis of pooled plasma samples individually analysed and included in the model (P 0.30 and P 0.46 respectively), and farm was not significant.

Table 2.5: Pooled plasma biotin analysis ANOVA general linear model

Variable	Adj SS	F	P	DF
Supplementation	2070	0.56	0.46	1
Heifer or Cow	16382	4.44	0.04*	1
Farm No	26671	1.81	0.14	4

(nmol/L) P* Significant

Milk quality records

Monthly milk records were collected on disk or in printed format and entered manually into a database using Microsoft Access for Windows '95, (Version 7, 1989-1995, Microsoft Corp.) from National Milk Records (NMR), (166 Hendford Hill, Yeovil, Somerset) or Milk Merit, (Frilsham Home Farm Business Units, Yattendon, Newbury) respectively. These records included total milk yield, compositional quality and cell counts. Farms 1, 2 and 5 were already enrolled with NMR with visits every month, and Farm 3 was monitored by Milk Merit on a monthly recording basis. Farm 4 was enrolled with NMR on a bi-monthly basis and was therefore, for the purpose of the trial, adjusted to monthly recordings at no cost to the farmer. The researcher's visit to collect milk samples was linked with the NMR and Milk Merit monthly visit on Farms 1 and 3, on the other farms the farmer preferred visits on separate days because of the lack of space in the milking parlour.

Lameness recording

One key objective of the trial was to identify every incident of lameness that occurred on the farms. To achieve this, the farmers were not charged when their cows were examined and treated by the veterinarian, when they suspected that a cow was lame.

Farmers contacted a trial veterinarian as soon as a cow was observed to be lame. The visiting veterinarian checked the cow was lame. The cow's foot was inspected by one of six trial veterinary surgeons and a form (Appendix I) was completed which recorded the date, farm, cow identification (freeze brand), lesion location, foot lesion type, treatment and vet identity. Additionally, 2+ photographs or slides were taken per foot. Each completed form represented one lame claw, which may have contained more than one lesion. In these cases all lesions were reported and where possible the veterinarian identified one lesion as causing lameness and others as secondary lesions. Therefore, all lesions were reported as a result of a lameness incident, some lesions may exist without causing lameness but these would not have been identified unless they accompanied a lesion that caused lameness. All data were recorded in Microsoft Access for Windows '95, (Version 7, 1989-1995, Microsoft Corp.) and included in the final analysis.

The lesions listed on the diagnosis of reporting form were chosen on the basis of previous studies highlighting common lesions and the observations of the participating veterinarians (Appendix I).

Locomotion assessment - The assessments were carried out on the lactating cows by the veterinarians on a lame or not lame basis. An initial assessment of the herd locomotion took place in the three weeks before the trial began on each farm, and was

then carried out bi-monthly. This procedure recorded the total number of cows, the number of lame cows and the cows not previously identified as lame by the farmer.

Any lame cows were examined by the veterinarian either immediately following the assessment, or within a few days.

Researchers, veterinarian and farmer meetings

Regular meetings were held approximately every three months, followed by an evening meal for all attending (Plate 2.5).

The session started with a meeting of the research team (VJH, LEG, AJP, RWB) and veterinarians (RWB, CW, GS, PC, CH, MH), the trial farmers and herdsmen (AW, AG, DK, SK, TD, NA, CC, PJ) joined at a later scheduled time (example of agenda can be seen in Appendix III). Minutes were taken from all meetings, actioned and used as a reference and record for further meetings for action and discussion details (example of minutes, Appendix III). These meetings were held to bring all of the interested parties together regularly throughout the trial so that any problems or queries could be raised, a successful method adopted in other studies (Green *et al* 1994; French *et al* 1994).

The veterinarian meeting took the form of an update on lameness and success of supplementation over the given period and discussions about individual farms and any problems encountered. The veterinarians discussed and standardised identification and reporting of different lesions. Identification of a specifically located lesion, which was considered to previously be misdiagnosed as a sole ulcer in a caudal site, was redefined by the veterinarians at one of the early meetings as a heel ulcer or necrotic heel track due to its aetiology and pathology. It was identified as a necrotic heel track by all veterinarians

in the trial as a result (Blowey *et al* 2000, Appendix VI). This process involved the use of forms and photographs of previously reported lesions to identify any necrotic heel tracks that had previously been identified as a sole ulcer. Updates on biotin research work and other research information relevant to the trial were also discussed.

The meetings assisted in encouraging the continued interest of the farmers without presenting any results on the effect of biotin. Farmers were presented with very basic overall lameness figures by farm. They predominantly used the time to ask questions of the veterinarians or research team about the trial, to discuss lameness, lesions and to interact with the other farmers and members involved in the trial. No lameness figures were described with relation to biotin supplementation and analysis was not carried out on the biotin effects until the trial was complete, to avoid bias. The final meeting was held once the trial was over to present the final results to the veterinarians and farmers involved.

When the researcher visited the individual farms before and following these meetings it was evident that they increased the farmers and herdsmen enthusiasm and aided in keeping the farmer confident in the process of the trial.

Data analysis

Frequency tables were run on all trial data to identify any mistakes or inconsistencies using Epi-info version 6.04 (Dean *et al* 1991) and distributions were identified using Minitab 10-5 (Minitab Inc.). Distributions, means and standard deviations were calculated and used to compare calving dates between biotin supplemented and unsupplemented animals to assess the success of the random allocation within farm using

Minitab 10-5 (Minitab Inc.). Univariate analysis was used on further data to detect the presence of crude significant differences on all recorded variables of the herd and management over the period of the trial and to detect significant differences in lameness and milk quality by biotin supplementation within farm and then over all farms together. Tests were carried out using 2 sample *t*-test, ANOVA general linear model and using the statistical program Minitab 10-5 (Minitab Inc.) and chi square analysis and frequency tables using Epi-info version 6.04 (Dean *et al* 1991) (Kirkwood 1988).

Lameness data were analysed further using multivariate techniques. Cox proportional hazard survival analysis, type III (Collett 1994) was applied to the data using Egret 2.0 (Cytel Inc. Cambridge MA). It was used to analyse the time to failure in days, using staggered entry times to the diagnosis of a specific lesion under analysis, or those individuals that contributed to the trial but were lost to follow up, to estimate the hazard ratio for an exposure to specific lesions and the influence of certain independent variables. Kaplan Meier curves were also plotted to show the pattern of the failure times of lesions over the time period of the trial and to identify any effect or interaction of biotin supplementation on this failure pattern. Biologically feasible interactions were tested and the model was also tested for goodness of fit (Collett 1994).

Plate 2.5: Meeting of the research team, farmers and herdsmen and veterinarians

CHAPTER THREE

RESULTS

Herd and farm

Different calving patterns on each of the five trial farms meant that the start times were staggered (Table 3.1). Herd size ranged from approximately 100 to 180 cows (Table 3.1). All farms remained in the trial for a full 18 months. The exact start and finish dates are shown in Table 3.1.

Table 3.1: Individual farm start and finish dates

FARM NUMBER	HERD SIZE (average)	START DATE	FINISH DATE
1	130	31/7/97	31/1/99
2	160	4/7/97	4/1/99
3	180	15/9/97	15/3/99
4	155	19/9/97	19/3/99
5	100	24/8/97	24/2/99

A total of 900 cows, 453 supplemented with biotin and 447 unsupplemented entered the trial (Table 3.2). There were approximately an equal number of animals with each supplementation (Table 3.2) indicating random allocation had successfully achieved a relatively equal distribution of numbers of cows to each supplementation.

No significant differences were found in the number of heifers or cows by supplementation (Table 3.3).

Table 3.2: Total number of cows that were included in the trial by farm and supplementation

	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	All farm total
Biotin Supplemented	83	90	113	108	59	453
Unsupplemented	84	90	114	107	52	447
Total	167	180	227	215	111	900

Table 3.3: Frequency of heifers and cows included in the trial by supplementation and farm.

		Farm					
Heifer/Cow	Biotin	1	2	3	4	5	Total
Heifer	Yes	34	28	44	52	25	183
	No	33	28	46	53	19	179
	Total	67	56	90	105	44	362
	P	0.95	0.87	0.93	0.95	0.66	0.97
Cow	Yes	49	62	69	56	34	270
	No	51	62	68	54	33	268
	Total	100	124	137	110	67	538
	P	0.95	0.87	0.93	0.95	0.66	0.97

*P significant (Yates corrected)

The biotin supplemented animals contributed a mean of 467 days (SE 6.8) to the trial and the unsupplemented animals 460 days (SE 7.0) which were not significantly different (P 0.44) *t*-test analysis (Table 3.4). No significant differences were found in time all cows contributed to the trial between the biotin supplemented and unsupplemented animals by farm (Table 3.5).

Table 3.4: Total days contributed by animals in the trial by biotin supplementation

	N	Mean	St Dev	SE Mean	P	DF	Q1	Q3
Biotin supplemented	453	457	158	7.5	0.54	896	298	577
Unsupplemented	447	451	159	7.5			287	577

* *P* significant, CI = 95% confidence interval

Animals that joined the trial at the beginning could potentially have two calves in the 18 months. There were 173 heifers that calved in the second season of the trial, of the total 900 animals, 88 were biotin supplemented and 85 unsupplemented. The number of calves on each farm or pooled were not significantly different between biotin supplemented and unsupplemented animals, except for Farm 4 ($P < 0.08$) for 1 and 2 calves born. More unsupplemented cows had one calf than the biotin supplemented and more biotin supplemented cows had two calves than the unsupplemented cows which may reflect fertility (Table 3.6).

Table 3.5: Days in the trial by individual farm and supplementation

Farm		N	Mean	St Dev	SE Mean	P (*t*)	Q1	Q3
1	Biotin supplemented	83	445	164	18.1	0.59	270	577
1	Unsupplemented	84	458	149	16.3		268	577
2	Biotin supplemented	90	495	143	15.0	0.98	459	581
2	Unsupplemented	90	494	148	15.6		521	581
3	Biotin supplemented	113	448	150	14.0	0.26	287	587
3	Unsupplemented	114	426	150	14.0		287	558
4	Biotin supplemented	108	449	177	17.0	0.78	298	577
4	Unsupplemented	107	442	176	17.0		298	577
5	Biotin supplemented	59	452	149	19.4	0.59	254	580
5	Unsupplemented	52	436	169	23.4		254	565

**P* significant, CI = 95% confidence interval

Table 3.6: Frequency of calves during the trial by farm and supplementation

		Farm					
No. Calves	Biotin	1	2	3	4	5	Total
0	Yes	2	4	5	12	0	23
	No	0	2	7	10	2	21
	Total	2	6	12	22	2	44
	P	0.24[a]	0.68[a]	0.78	0.82	0.22[a]	0.91
1	Yes	37	41	59	50	27	214
	No	35	38	60	64	21	218
	Total	72	79	119	114	48	432
	P	0.82	0.76	0.94	0.08	0.70	0.69
2	Yes	44	45	49	46	32	216
	No	49	50	47	33	29	208
	Total	93	95	96	79	61	424
	P	0.63	0.58	0.99	0.08	0.66	0.66

*P significant (Yates corrected, [a] 2-tailed fishers exact)

The number of days after the start of the trial each individual calved for the first time was also estimated and analysed (Table 3.7). Stratification by predicted calving date was successful, although, Farm 3 had a greater mean number of days to first calving in the unsupplemented cows than the biotin supplemented cows (P 0.06) and may reflect fertility.

Table 3.7: Days from trial entry to first calving by farm and supplementation (Kruskal-Wallis test)

Farm	Biotin	N	Median	Average rank	Z	P
1	Yes	83	67.0	82.4	-0.43	0.66
	No	84	79.0	85.6	0.43	
2	Yes	90	52.5	91.0	0.13	0.90
	No	90	51.0	90.0	-0.13	
3	Yes	113	84.0	105.3	-1.88	0.06
	No	113	108.0	121.7	1.88	
4	Yes	108	62.0	101.1	-1.62	0.10
	No	107	82.0	114.9	1.62	
5	Yes	59	84.0	60.7	1.64	0.10
	No	52	69.5	50.7	-1.64	

*P significant (Yates corrected)

The reasons given for animals leaving the trial prior to the finish date included, death, culling and sale. These were recorded but the reasons were not commonly recorded. Table 3.8 shows the frequency of culling by farm and supplementation. The trial took place at a time when farms culled animals with tuberculosis (TB). Most of the cows that required culling as a result of TB were identified at the beginning of the trial and were therefore not included, however, some of the trial cows became positive reactors during the period of the trial and were culled. Farm 5, a small farm (approximately 100 cows), had a high rate of culling and the majority was attributed to

TB (Table 3.8). No significant difference in culling between the biotin supplemented and unsupplemented cows were found.

Table 3.8: Frequency of animals that left the trial as a result of culling, by farm and supplementation

	Farm					
Biotin	1	2	3	4	5	Total
Yes	9	10	7	15	12	53
No	10	7	5	21	19	62
Total	19	17	12	36	31	115
P	0.97	0.61	0.75	0.34	0.09	0.38

* P significant (Yates corrected)

A very few cows died during the trial, causes included:- accidental injury and calving complications (Table 3.9). No significant difference in death rate was found overall.

Table 3.9: Frequency of animals that left the trial as a result of death, by farm and supplementation

	Farm					
Biotin	1	2	3	4	5	Total
Yes	2	0	4	2	0	8
No	2	0	2	3	1	8
Total	4	0	6	5	1	16

* P significant (Yates corrected)

A total of 179 cows were sold from the five farms (Table 3.10). There were no significant differences between the number of cows sold by supplementation.

Table 3.10: Frequency of animals that left the trial as a result of sale, separated by supplementation

	Farm					
Biotin	1	2	3	4	5	Total
Yes	16	16	19	15	14	80
No	18	15	26	22	18	99
Total	34	31	45	37	32	179
P	0.88	1.00	0.33	0.26	0.90	0.11

*P significant (Yates corrected)

The lactation number of individual cows was recorded at the end of the trial (Table 3.11). A difference in lactation number by farm was found, for example, Farms 2, 3 and 5 had some cows reaching their 10^{th} - 12^{th} lactation and Farm 4 did not have any cows beyond their 7^{th} lactation. Chi square analysis showed that the individual lactation cows were evenly represented in supplemented and unsupplemented groups by individual farms.

Table 3.11: Frequency of lactation number by farm and supplementation

Farm	Biotin	Lactation No											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Yes	21	20	22	15	2	0	2	0	1	0	0	0
	No	17	20	24	16	2	3	1	1	0	0	0	0
	Total	38	40	46	31	4	3	3	1	1	0	0	0
	P	0.55	0.89	0.90	0.97	1.00[a]	0.24[a]	0.62[a]	1.00[a]	0.50[a]	0	0	0
2	Yes	14	23	17	13	9	4	6	0	0	1	2	0
	No	13	23	17	12	12	6	5	1	0	0	0	1
	Total	27	46	34	25	21	10	11	1	0	1	2	1
	P	1.00	0.86	0.85	1.00	0.64	0.74	1.00	1.00[a]	0	1.00[a]	0.50[a]	1.00[a]
3	Yes	32	21	21	15	13	5	0	3	1	1	1	0
	No	35	17	23	15	14	4	3	2	1	0	0	0
	Total	67	38	44	30	27	9	3	5	2	1	1	0
	P	0.80	0.57	0.89	0.86	0.98	0.75	0.25[a]	0.68[a]	1.00[a]	0.50[a]	0.50[a]	0
4	Yes	34	24	18	14	9	6	3	0	0	0	0	0
	No	42	22	13	10	15	5	0	0	0	0	0	0
	Total	76	46	31	24	24	11	3	0	0	0	0	0
	P	0.29	0.89	0.44	0.53	0.27	0.99	0.25[a]	0	0	0	0	0
5	Yes	13	14	8	6	7	3	4	1	2	1	0	0
	No	10	16	2	7	6	6	0	1	4	0	0	0
	Total	23	30	10	13	13	9	4	2	6	1	0	0
	P	0.90	0.53	0.10[a]	0.81	0.81	0.30[a]	0.12[a]	1.00[a]	0.42[a]	1.00[a]	0	0

*P significant (Yates corrected, [a] 2-tailed fishers exact)

In conclusion an even representation of animals in the population within and between farms was maintained across those supplemented with biotin and those unsupplemented.

Biotin supplementation systems

The number of doses of biotin per milking is presented in Figures 3.1 to 3.5. On Farm 1 (Figure 3.1) the supplementation process was quite inconsistent throughout the trial, this was largely thought to be due to staff error during milking. On Farm 2 (Figure 3.2) it was evident that there were some specific areas where doses were missing from the chart, specifically in November 1998, this corresponds with a problem that was encountered with the dispensing system where fluid had escaped into the electrical junction outside the tank and repeatedly 'tripped' the system.

The cows on Farms 3 (Figure 3.3) and 4 (Figure 3.4) damaged the system by pulling piping away from the wall. On Farm 3 parts of the system had to be replaced. This occurred in August 1998 on Farm 3 and March 1998 on Farm 4, and replaced immediately. The increase in doses observed at the end of the trial in Figure 3.3 on Farm 3 was a result of system error as the system was not functional at this time.

Farm 4 also experienced a power surge which damaged the computer early in the trial, before the first recordings on Figure 3.4, which resulted in mains protection devices being installed in all five systems.

Farm 5 experienced some problems in the circuit of the system around the end of December 1998 (Figure 3.5), the safety system cut the power to the whole supplementation system. Fluctuating levels observed in Figures 3.1 to 3.5 represent the seasonal effect from calving.

There were a few minor problems with the dispensing systems over the period of the trial which are highlighted in the previously detailed graphs and commonly rectified successfully and mostly immediately. However, it can be seen from the majority of the farm's graphs and especially Farm 5, that supplementation was

consistent and the patterns and figures corresponds with the amount of cows in milk at that time, with the exception of Farm 1.

The excessively high readings in the first four months of 1998 in all five graphs represents the researcher monitoring biotin supplementation dose. Later checks were removed from the data by keypad codes, only available to the researcher and system designer.

Otherwise, the cows readily consumed their feed ration in the parlour during the winter, however, during the summer some feed was observed in a few individual feed troughs after milking indicating that some of the cows were not consuming all of the pellets. This occurred occasionally on all farms but most predominantly on Farm 3, where a more mineral based feed was used in the parlour. Troughs were cleaned out before the next milking.

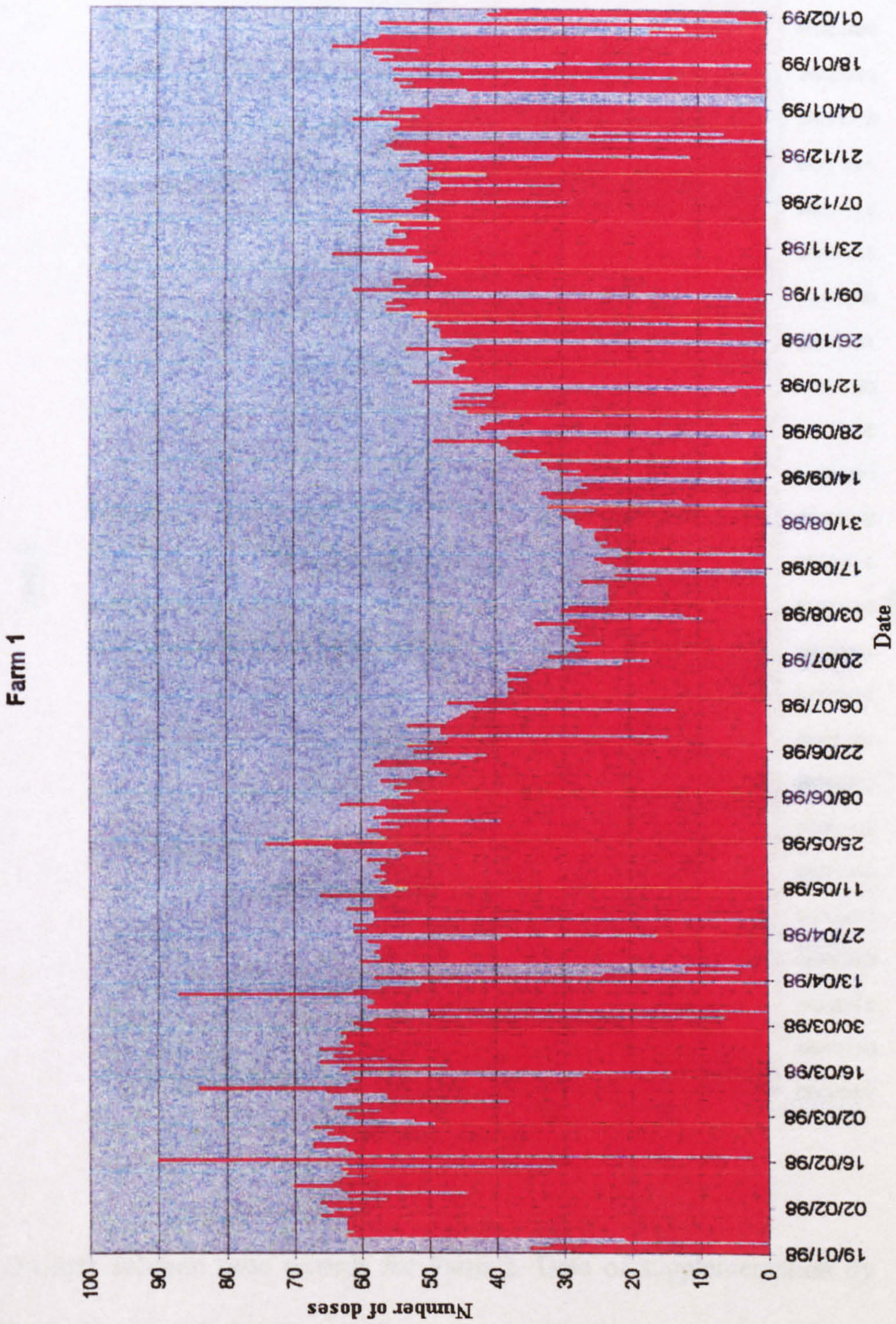


Figure 3.1: Parlour solution dose records for Farm 1. Date of supplementation by number of doses of biotin solution dispensed

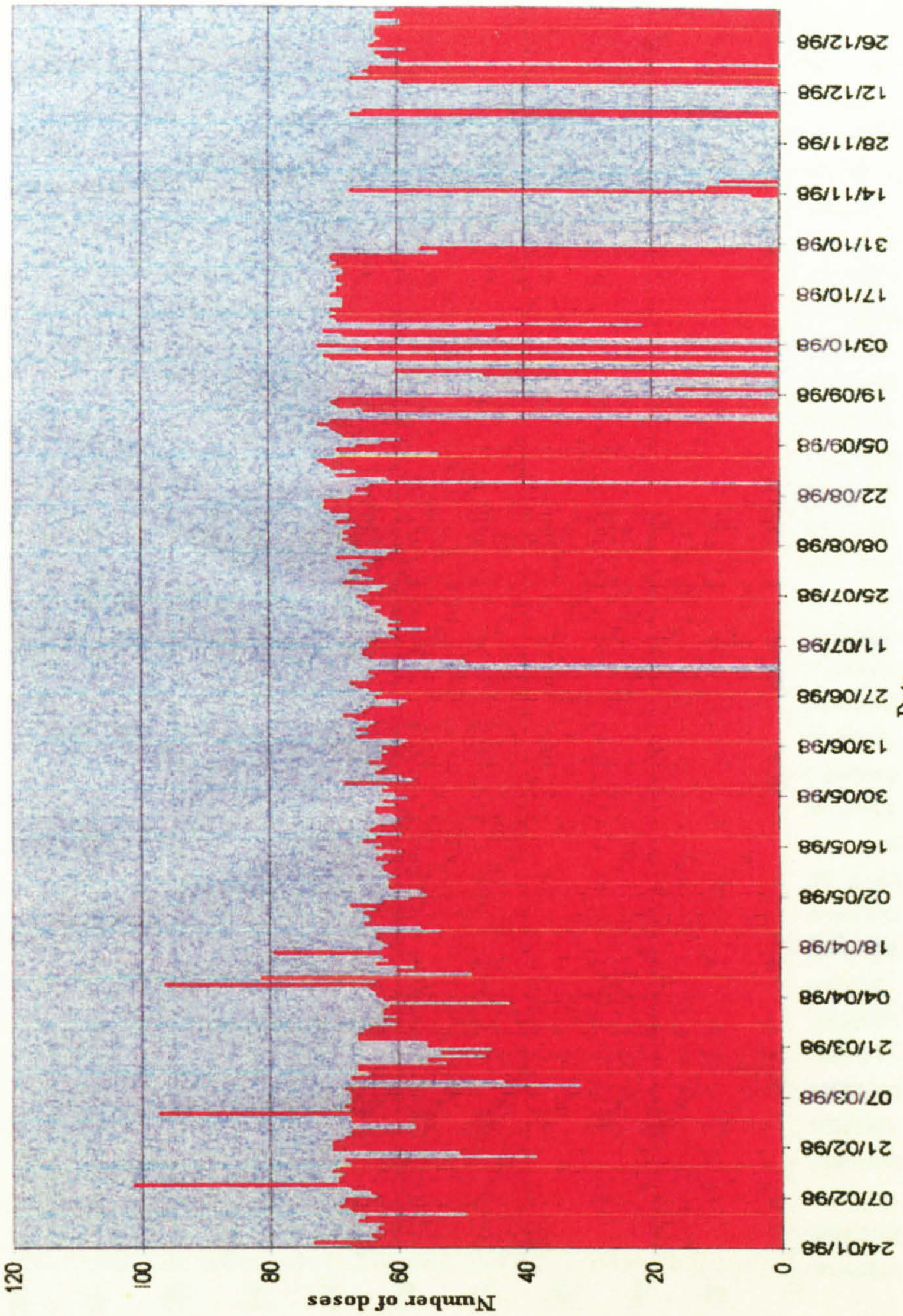


Figure 3.2: Parlour solution dose records for Farm 2. Date of supplementation by number of doses of biotin solution dispensed.

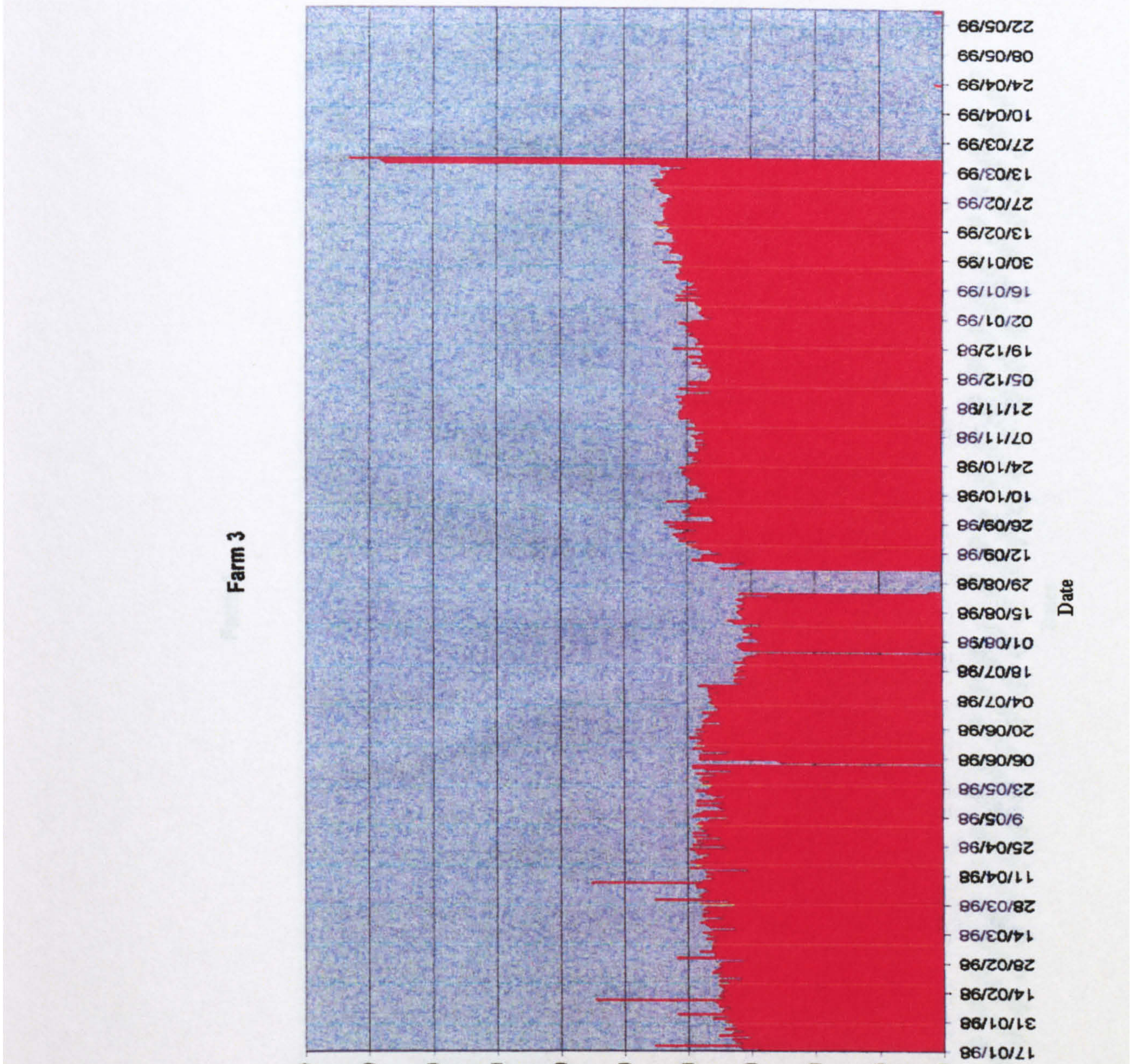


Figure 3.3: Parlour solution dose records for Farm 3. Date of supplementation by number of doses of biotin solution dispensed.

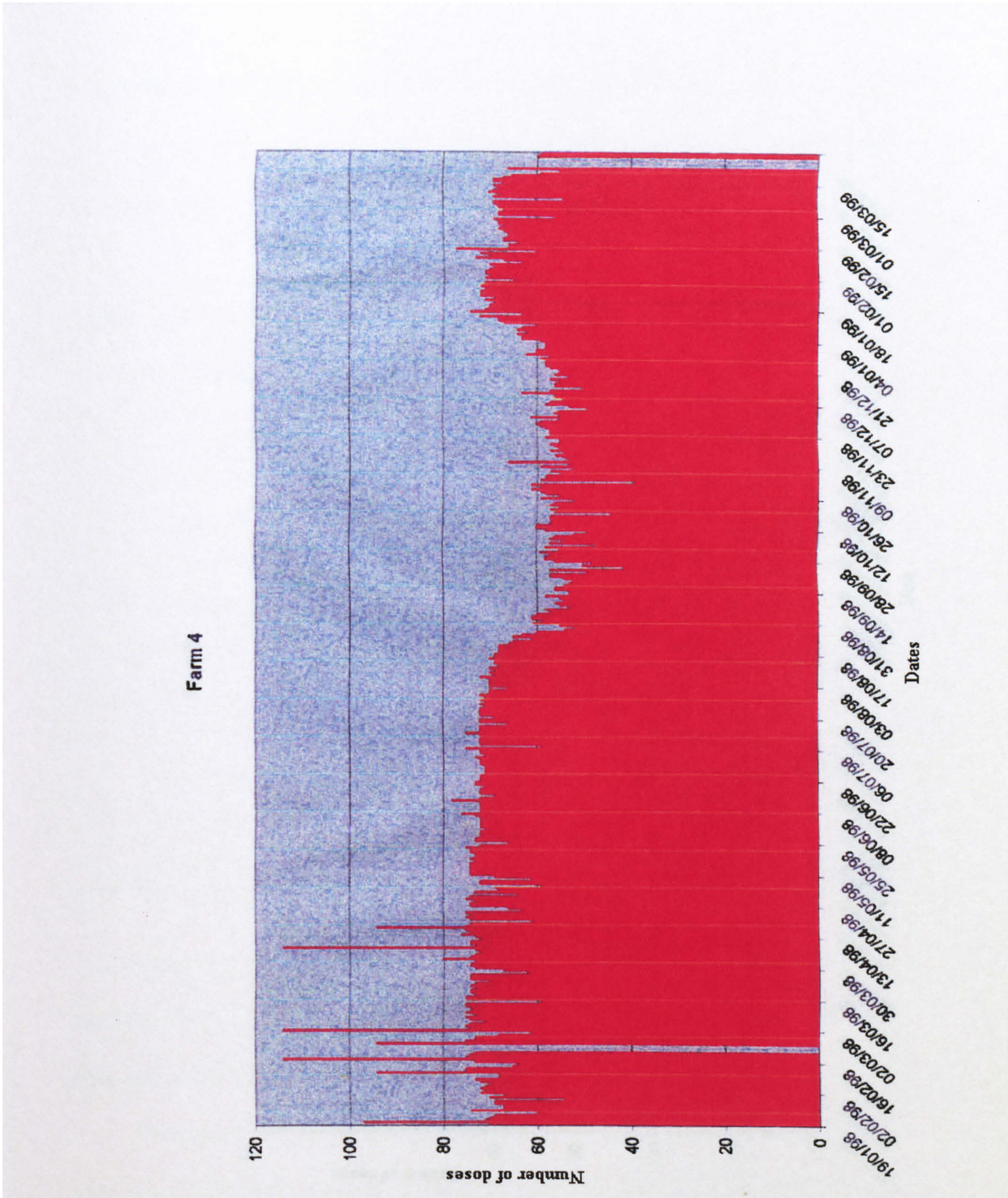


Figure 3.4: Parlour solution dose records for Farm 4. Date of supplementation by number of doses of biotin solution dispensed.

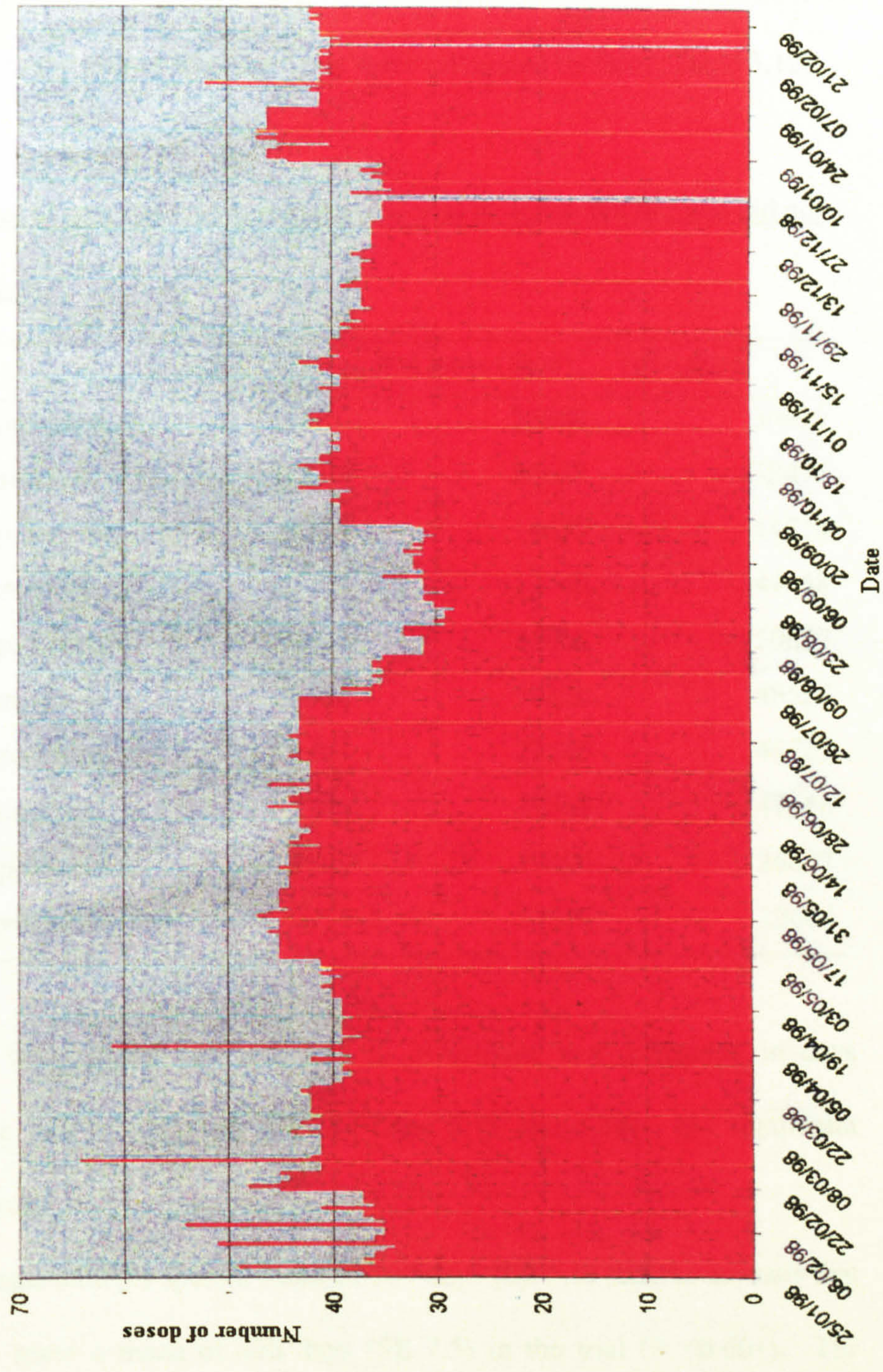


Figure 3.5: Parlour solution dose records for Farm 5. Date of supplementation by number of doses of biotin solution dispensed.

Lameness data

The number of days contributed by cows or heifers that became lame at least once in the period of the trial or were not lame were calculated by farm (Table 3.12).

Table 3.12: Number of cow days contributed to the trial by those that did and did not become lame by farm

Farm		Lame (days)	Not lame (days)	Total days
1	Biotin supplemented	10981	25958	36939
	Unsupplemented	11817	26658	38475
2	Biotin supplemented	29879	14635	44514
	Unsupplemented	30719	13749	44468
3	Biotin supplemented	19623	31004	50627
	Unsupplemented	18308	30220	48528
4	Biotin supplemented	25984	22545	48529
	Unsupplemented	27783	19563	47346
5	Biotin supplemented	7203	19494	26697
	Unsupplemented	7870	14814	22684

The data were pooled and analysed to investigate any difference in days contributed to the trial by supplementation (Table 3.13 and 3.14). No significant difference was found.

Overall, lame animals spent a mean of 509 days (SE 5.5) and the animals that were never lame spent a mean of 420 days (SE 7.5) in the trial ($P < 0.001$). The number of animals involved in this analysis was more evenly proportioned than analysis on individual farms (Table 3.15).

Table 3.13: Number of days in the trial contributed by cows that became lame, by supplementation

Biotin	N	Mean*	St Dev	SE Mean	P	DF
Supplemented	251	511	119	7.5	0.67	497
Unsupplemented	250	507	126	8.0		

* *P* significant, CI = 95% confidence interval

Table 3.14: Number of days in the trial contributed by cows that were never lame, by supplementation

Biotin	N	Mean	St Dev	SE Mean	P	DF
Supplemented	267	426	172	11	0.41	518
Unsupplemented	254	413	169	11		

* *P* significant, CI = 95% confidence interval

Table 3.15: Number of days in the trial contributed by cows that did and did not become lame, pooled

	N	Mean	St Dev	SE Mean	P	DF
Non lame cows	521	420	170	7.5	<0.001*	946
Lame cows	501	509	123	5.5		

* *P* significant, CI = 95% confidence interval

A significant difference was also found on Farms 2, 4 and 5 and marginal of Farms 1 and 3 once the data was separated by farm (Table 3.16).

Table 3.16: Number of days in the trial contributed by cows that did and did not become lame by individual farm

Farm		N	Mean	St Dev	SE Mean	P (*t*)	DF
1	Not lame	120	438	163	15	0.06	101
	Lame	47	485	134	20		
2	Not lame	67	424	183	22	<0.001*	87
	Lame	113	536	95	8.9		
3	Not lame	145	422	152	13	0.09	163
	Lame	78	458	145	16		
4	Not lame	109	386	193	18	<0.001*	147
	Lame	232	521	118	7.8		
5	Not lame	80	429	167	19	0.05*	72
	Lame	31	486	125	22		

**P* significant, CI = 95% confidence interval

Initially a comparison of all diagnosed lameness was made by farm and supplementation. The lesions included were classified as lameness-causing or primary lesions (highlighted on the reporting form by the veterinarian). Secondary lesions were noted or lesions that were present in the same foot or claw as the primary lesion but was not the cause of lameness. Appendix IV lists the secondary lesions identified during the trial. The highest incidence of 14 lesions was under run sole, 6.07 per 100 cows per year, this commonly accompanied sole ulcer. A significant difference was observed between the biotin supplemented (0.70 cases per 100 cows per year) and unsupplemented cows (2.35 cases per 100 cows per year) for under-run sole (P 0.04). No significance was observed between the biotin supplemented and unsupplemented cows with the other secondary lesions.

Table 3.17 shows the incidence of lameness on all five farms and pooled per 100 cows per year. The total incidence rate for the five farms regardless of supplementation was 68.9 per 100 cows per year (Table 3.17). This analysis included all lameness data which included the 'other' diagnosis category detailed on the recording form (Appendix I), which is reported later in this chapter and includes 30 separate lesions which did not always involve claw lesions. Farm 2 had the largest incidence of lameness of 111.5 per 100 cows per year. Farm 5 had a comparatively low incidence of lameness 32.5 per 100 cows per year and was the only farm that had a significant difference between supplemented and unsupplemented cows; 27.3 per 100 cows per year and 38.6 per 100 cows per year respectively (P 0.003, Table 3.17).

Table 3.17: Incidence (per 100 cows per year) of all causes of lameness by farm and supplementation

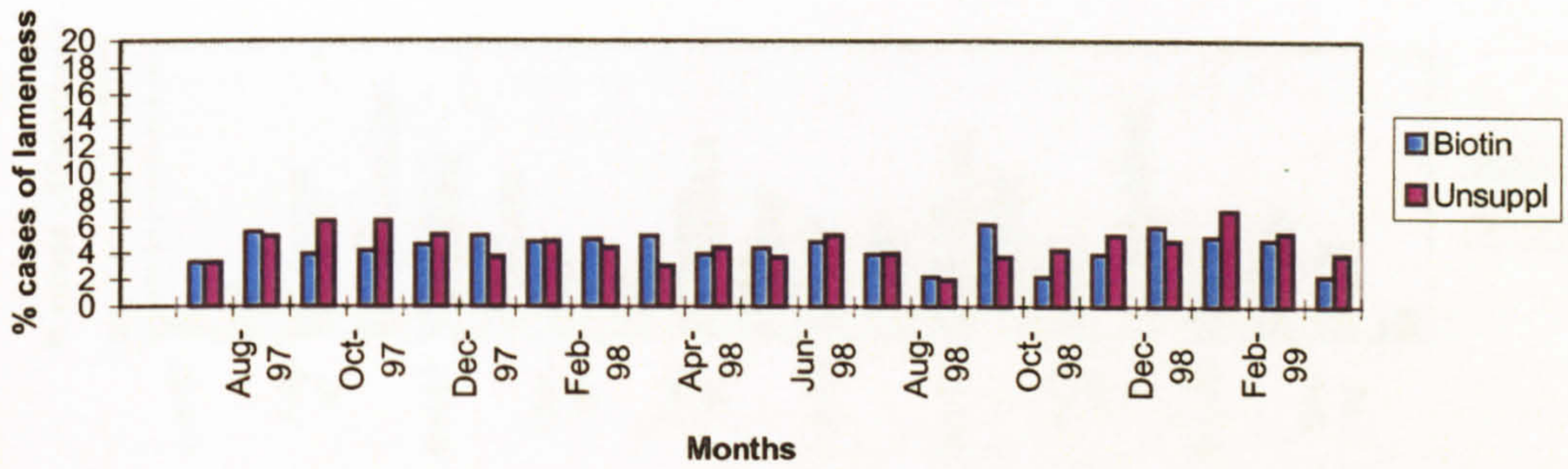
Farm ID	1	2	3	4	5	Total
Biotin supplemented	32.6	108.2	51.2	91.0	27.3	66.4
Unsupplemented	30.4	114.9	64.7	85.6	38.6	71.2
P	0.70	0.30	0.1	0.57	0.003*	0.65
Total incidence	31.6	111.5	57.9	88.8	32.5	68.9
Number of cattle	167	180	227	215	111	900

**P* Significant

The lameness data were plotted by calendar month of the trial to investigate any seasonal effect (Figure 3.6). The amount of lameness observed in June to September 1997 and January to April 1999 did not include lameness from all five farms as the farms started and finished at staggered times. A slight increase in overall lameness was found in the housing period of October 1997 to February 1997 and

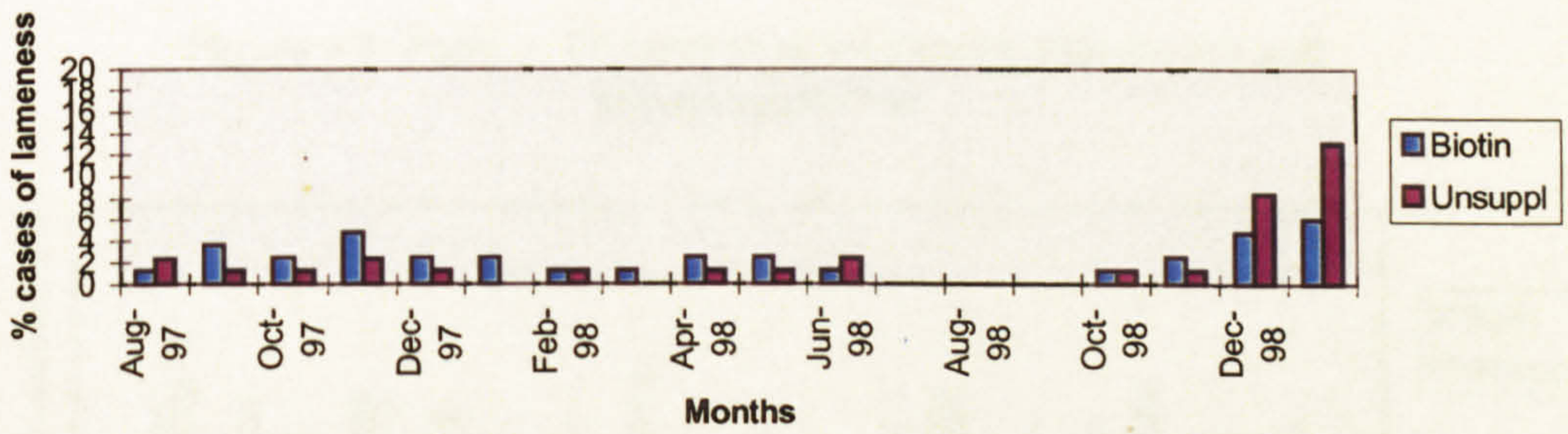
increased slightly in September and October 1998 and decreasing mainly in August 1998.

Figure 3.6: All farms; Overall lameness lesions by month of the trial and supplementation



There were no large differences between the seasonal periods. Farm 1 (Figure 3.7) had an overall incidence of 31.6 per 100 cows per year, the lowest incidence of the five farms and did not have any lameness during the summer months of July to September 1998. The unsupplemented animals had an increase in lameness in the final months of the trial (December 1998 and January 1999).

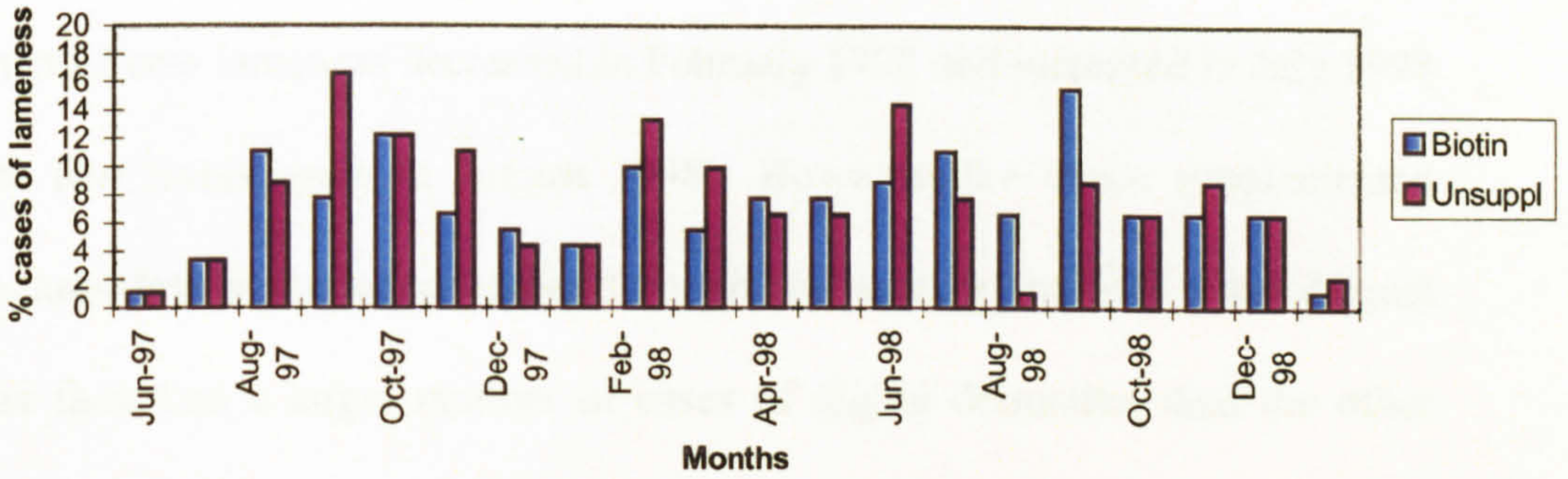
Figure 3.7: Farm 1; All lameness lesions over the trial months by supplementation



Farm 2 (Figure 3.8) had a similar pattern of lameness over the months of the trial, with no definite seasonal pattern. There was an increase in lameness from

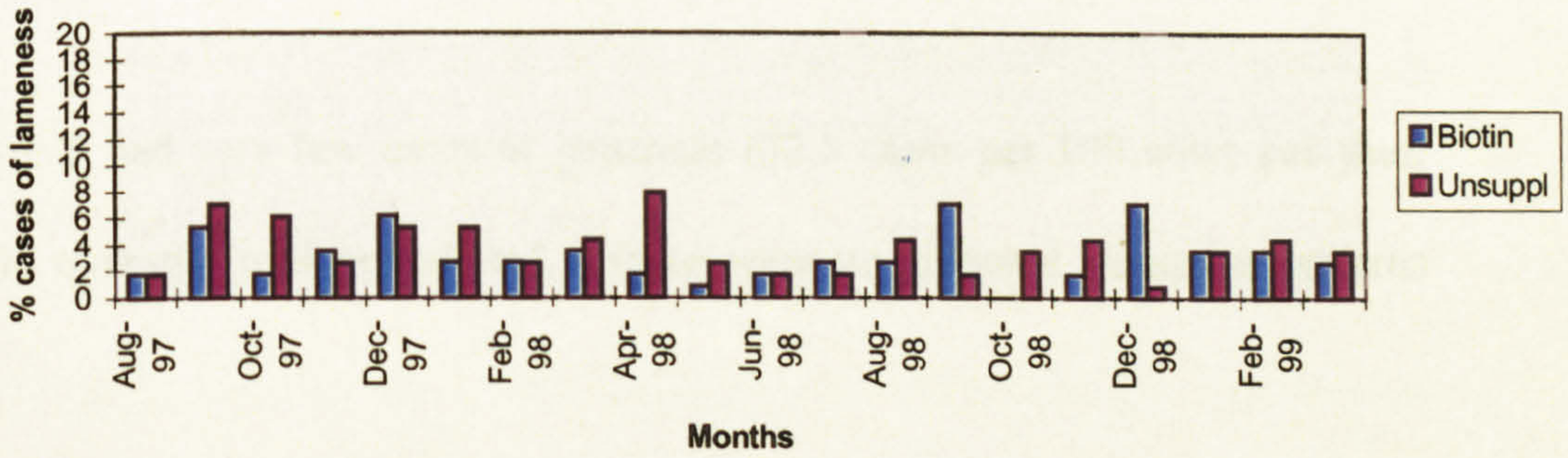
August 1997 when sharp flint-like stones were put on the surface of the entrance to the yard and milking parlour.

Figure 3.8: Farm 2; All lameness lesions by month of the trial and supplemenation



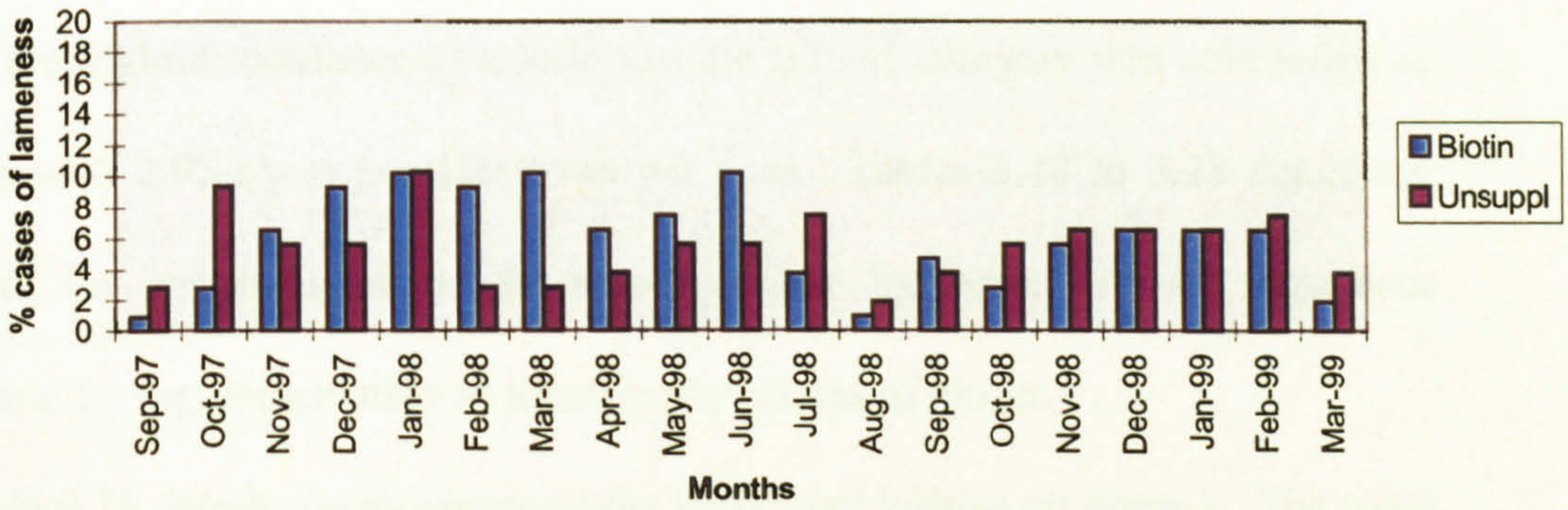
Farm 3 (57.9 cases per 100 cows per year, Table 3.17) had a lower proportion of lameness cases in the summer months and an increase in cases around housing in September and October 1997/98 (Figure 3.9). In April 1998 there were greater proportions of cows lame that were unsupplemented and a greater lameness in the biotin supplemented cows compared to the unsupplemented cows in September and December 1998. No significant effect of season was observed.

Figure 3.9: Farm 3; All lameness lesions by trial month and supplementation



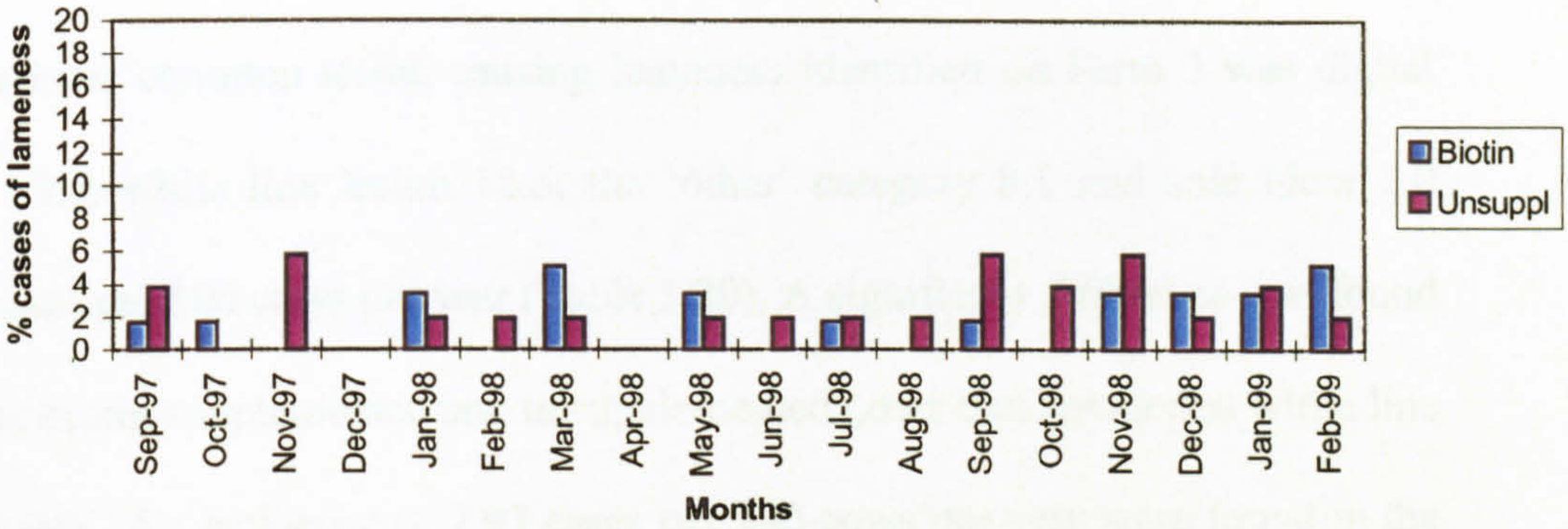
A more definite pattern was observed on Farm 4 (incidence of 88.8 cases per 100 cows per year, Table 3.17) with an increase in lameness around the housing period of October 1997 and September/October 1998 (Figure 3.10). However, lameness did not decrease in the summer months until August 1998. In the unsupplemented cow lameness decreased in February 1998 and increased in July 1998 to return to low levels again in August 1998. However, the biotin supplemented cow's lameness levels remained higher through the summer months until August 1998. This farm had a larger number of cases of digital dermatitis than the other farms and occurrence over time will be discussed later in this chapter.

Figure 3.10: Farm 4; All lameness lesions by trial month and supplementation



Farm 5 had very few cases of lameness (32.5 cases per 100 cows per year, Table 3.17) over the trial period and a there were no seasonal lameness patterns (3.11).

Figure 3.11: Farm 5; All lameness lesions by trial month and supplementation



The lameness reporting form included 13 lesions to choose from and an 'other' category where the alternative lesions were detailed in an allocated space. Appendix IV details the 30 'Other' lesions which collectively represented a high incidence on particular farms shown in the following Tables 3.18 to 3.23. Once the lesions were identified, the highest incidence of a lesion in the 'other' category was sole ledge or overgrowth with 2.95 cases per 100 cows per year. Tables 3.18 to 3.23 detail the incidence of the lesions listed on the standard form by farm, with all farm data combined and by supplementation to identify any effects of biotin.

Table 3.18 details the incidence of the individual lesions on Farm 1. The most common lesions identified on Farm 1 were interdigital necrobacillosis lameness 11.1, sole ulcer lameness 3.9, the 'other' category 3.4 and white line lesion lameness 2.9 cases per 100 cows per year. None of the lesions on this farm were significantly different between biotin supplemented and unsupplemented cows.

On Farm 2, sole ulcer 32.0, white line lesion 29.1, heel ulcer 19.7 and foreign body lameness 6.6 cases per 100 cows per year were most commonly diagnosed (Table 3.19). A significant difference was found between the biotin supplemented and

unsupplemented dairy cows with foreign body penetration lameness (P 0.003). No significant difference in supplementation was observed in the remaining lesions.

The most common lesion causing lameness identified on Farm 3 was digital dermatitis 13.6, white line lesion 12.5, the 'other' category 8.1 and sole ulcer 7.0 lameness cases per 100 cows per year (Table 3.20). A significant difference was found between the biotin supplemented and unsupplemented cows that developed white line lesion (P 0.04). An incidence of 7.93 cases per 100 cows per year were found in the biotin supplemented cows and 17.30 cases per 100 cows per year in the unsupplemented cows.

Table 3.18: Incidence (cases per 100 cows per year) of lameness on Farm 1 by supplementation

Factor	Biotin Supplemented	Unsupplemented	P	Total
Sole Ulcer	4.94	2.85	0.49	3.87
White line	1.98	3.79	0.68	2.90
Digital Dermatitis	1.98	2.85	1.00	2.42
Interdigital necrobacillosis	10.87	11.38	0.97	11.13
Foreign body	1.98	0	0.24	0.97
Horizontal wall fissure	0	0	0	0
Interdigital growth	0	0.95	1.00	0.48
Heel ulcer	3.95	0.95	0.21	2.42
Under run wall	0	0	0	0
Under run sole	0	0.95	1.00	0.48
Slurry heel	0	0	0	0
Sole haemorrhage	1.98	1.90	1.00	1.93
Vertical wall fissure	0	0	0	0
Other	3.95	2.85	0.72	3.39

*P significant (2-tailed fisher exact)

Farm 4 had a particularly high rate of digital dermatitis in comparison with the other farms, 32.7 cases per 100 cows per year. However, when the figures were analysed by biotin supplement no significant difference was observed (P 0.93) (Table 3.21).

The most common lesions on Farm 4 following digital dermatitis (Table 3.21) were sole ulcer 15.2, interdigital necrobacillosis 12.2 and the 'other' category 10.3 cases per 100 cows per year. None of the lameness causing lesions displayed a significant difference when compared by supplementation.

Table 3.19: Incidence (cases per 100 cows per year) of lameness on Farm 2 by supplementation

Factor	Biotin Supplement	Unsupplemented	P	Total
Sole Ulcer	32.8	31.19	0.88	31.99
White line	24.60	33.65	0.13	29.12
Digital Dermatitis	1.64	3.28	0.68	2.46
Interdigital necrobacillosis	6.56	4.10	0.56	5.33
Foreign body	1.64	11.49	0.003*	6.56
Horizontal wall fissure	0	0	0	0
Interdigital growth	0	0	0	0
Heel ulcer	22.14	17.24	0.40	19.69
Under run wall	0	0	0	0
Under run sole	4.10	3.28	1.00	3.69
Slurry heel	0	0.82	1.00	0.41
Sole haemorrhage	5.74	3.28	0.53	4.51
Vertical wall fissure	0.82	1.64	1.00	1.23
Other	5.74	2.46	0.33	4.10

*P Significant (Yates corrected)

Farm 5 had one of the lowest lameness incidences overall (Table 3.22). The most common cause of lameness was white line lesion at a relatively low rate of 8.1, followed by sole ulcer 7.4, foreign body 3.7 and heel ulcer and the 'other' category both with 3.0 cases per 100 cows per year (Table 3.22). There was no significant difference by lesion between cows that were supplemented with biotin and unsupplemented cows. Many of the possible lesions were not observed on this farm at all during the period of the trial.

Table 3.20: Incidence (cases per 100 cows per year) of lameness on Farm 3 by supplementation

Factor	Biotin Supplement	Unsupplemented	P	Total
Sole Ulcer	5.05	9.02	0.35	6.99
White line	7.93	17.30	0.04*	12.51
Digital Dermatitis	11.53	15.79	0.49	13.62
Interdigital necrobacillosis	4.32	3.01	0.54[a]	3.68
Foreign body	2.16	3.76	0.72[a]	2.94
Horizontal wall fissure	3.60	0.75	0.12[a]	2.21
Interdigital growth	1.44	0	0.25[a]	0.74
Heel ulcer	1.44	0	0.25[a]	0.74
Under run wall	0	0	0	0
Under run sole	0	3.01	0.12[a]	1.47
Slurry heel	0	0	0	0
Sole haemorrhage	2.16	0	0.12[a]	1.10
Vertical wall fissure	1.44	0.75	0.62[a]	1.10
Other	7.21	9.02	0.84	8.10

*P Significant (Yates corrected, [a] 2-tailed fisher exact)

Table 3.21: Incidence (cases per 100 cows per year) of lameness on Farm 4 by supplementation

Factor	Biotin Supplement	Unsupplemented	P	Total
Sole Ulcer	18.80	11.56	0.12	15.23
White line	7.52	7.71	0.83	7.61
Digital Dermatitis	33.09	32.38	0.93	32.74
Interdigital necrobacillosis	11.28	13.10	0.82	12.18
Foreign body	2.26	0.77	0.62 [a]	1.52
Horizontal wall fissure	0	0	0	0
Interdigital growth	3.76	3.85	1.00 [a]	3.81
Heel ulcer	1.50	3.08	0.44 [a]	2.28
Under run wall	0.75	0.77	1.00 [a]	0.76
Under run sole	5.26	2.31	0.33 [a]	3.81
Slurry heel	0.75	0	1.00 [a]	0.38
Sole haemorrhage	0.75	2.31	0.37 [a]	1.52
Vertical wall fissure	0	0	0	0
Other	9.02	11.56	0.66	10.28

*P Significant (Yates corrected, [a] 2-tailed fisher exact)

Table 3.22: Incidence (cases per 100 cows per year) of lameness on Farm 5 by supplementation

Factor	Biotin Supplement	Unsupplemented	P	Total
Sole Ulcer	5.47	9.65	0.51 [a]	7.39
White line	5.47	11.26	0.39	8.13
Digital Dermatitis	0	0	0	0
Interdigital necrobacillosis	2.73	0	0.50 [a]	1.48
Foreign body	2.73	4.83	0.66 [a]	3.69
Horizontal wall fissure	0	0	0	0
Interdigital growth	0	0	0	0
Heel ulcer	1.37	4.83	0.34 [a]	2.96
Under run wall	0	0	0	0
Under run sole	0	0	0	0
Slurry heel	0	1.61	0.47 [a]	0.74
Sole haemorrhage	1.37	1.61	1.00 [a]	1.48
Vertical wall fissure	0	0	0	0
Other	5.47	0	0.12 [a]	2.96

*P Significant (Yates corrected, [a] 2-tailed fisher exact)

The above farm data were pooled and overall figures for diagnosed lesions and any effect of biotin supplementation examined (Table 3.23). Only one lesion showed a significant difference between the biotin supplemented cows and unsupplemented cows and this was white line lesion with 10.0 and 15.4 cases per 100 cows per year respectively. The other common lameness lesions included sole ulcer 13.8, digital dermatitis 12.0 and interdigital necrobacillosis 7.1 cases per 100 cows per year.

Table 3.23: Incidence (cases per 100 cows per year) of lameness lesions on all farms combined by supplementation

Factor	Biotin Supplement	Unsupplemented	P	Total
Sole Ulcer	14.26	13.40	0.66	13.84
White line	10.03	15.40	0.01*	12.68
Digital Dermatitis	11.27	12.68	0.58	11.96
Interdigital necrobacillosis	7.39	6.88	0.77	7.14
Foreign body	2.11	4.17	0.08	3.12
Horizontal wall fissure	0.88	0.18	0.22[a]	0.53
Interdigital growth	1.23	1.09	0.98	1.16
Heel ulcer	6.34	5.25	0.47	5.80
Under run wall	0.18	0.18	1.00[a]	0.18
Under run sole	2.11	2.18	0.86	2.14
Slurry heel	0.18	0.36	0.62[a]	0.27
Sole haemorrhage	2.46	1.81	0.56	2.14
Vertical wall fissure	0.53	0.54	1.00[a]	0.53
Other	6.51	5.98	0.75	6.25

*P Significant (Yates corrected, [a] 2-tailed fisher exact)

To investigate seasonal patterns with the most common lesions observed in the trial, data of, sole ulcer, white line, digital dermatitis and interdigital necrobacillosis, from all of the five farms were pooled and percentage cases of the lesions were plotted according to the month in which they occurred. The occurrence of these lesions following calving was also calculated and plotted. These figures apply to both first and second calving dates and include lesions up to 52 weeks after calving when a second calving did not occur.

Figure 3.12 shows the pattern of sole ulcer lesion lameness over the trial, which was observed mainly in the biotin supplemented cows during the housing

period of 1997/98 but was not as high in the following winter housing period 1998/99. A definite pattern was not observed between the biotin supplemented and unsupplemented cows.

Figure 3.13 shows that the greatest amount of lameness associated with sole ulcer was encountered predominantly between week 10 and 15 after calving.

Figure 3.12: Sole ulcer cases by trial month and supplementation

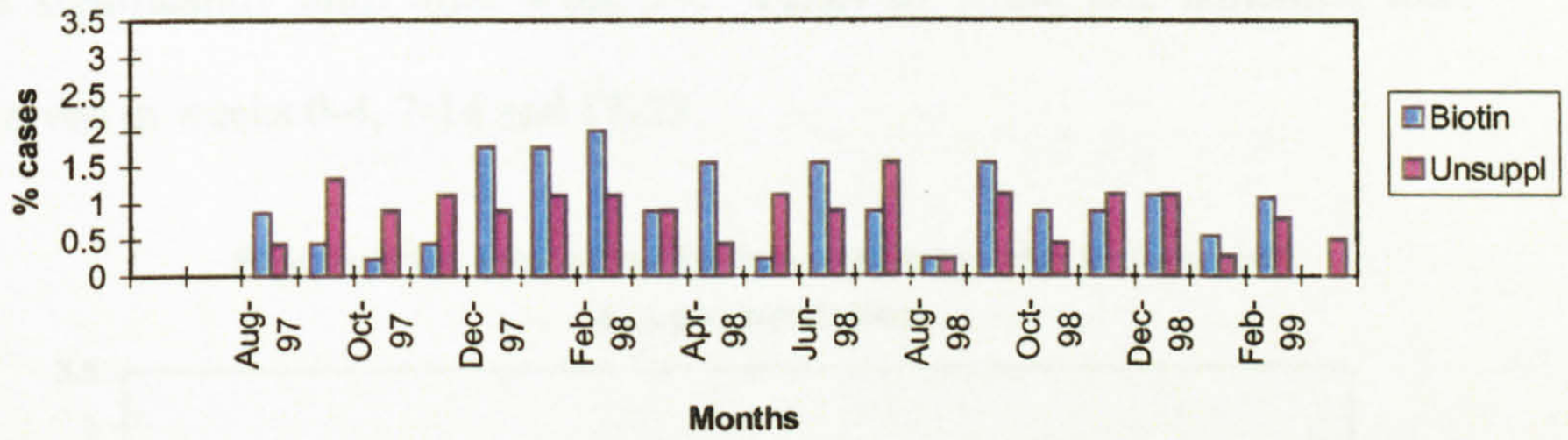
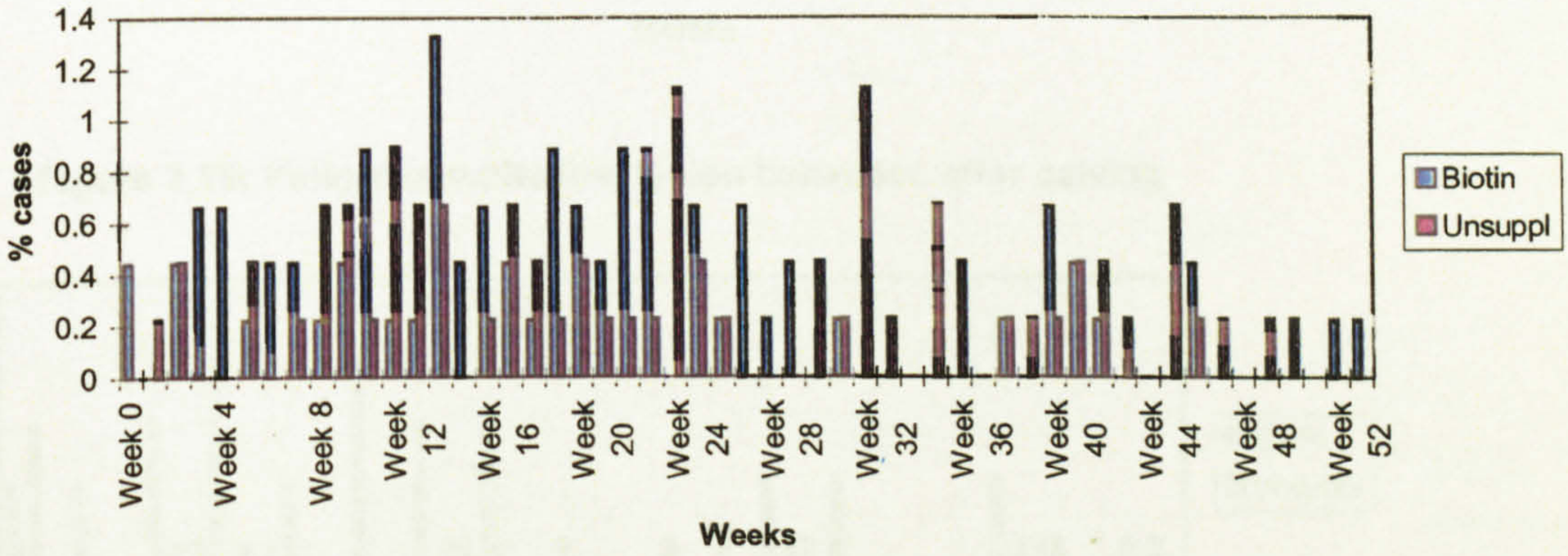


Figure 3.13: Pattern of sole ulcer lameness after calving



It is clearly observed in Figure 3.14 that white line lesion lameness was more common in the unsupplemented cows. A higher amount of cases was observed in the

unsupplemented cows in September and October 1997, February 1998 (housing period), June 1998 (grazing turnout) and October 1998 (start of housing). A slightly higher amount of white line lesion lameness was observed in the biotin supplemented cows in August 1997 and September 1998 compared with the unsupplemented cows. In the weeks after calving it appeared that white line lesion caused lameness fairly early after the calving date (Figure 3.15). The highest proportion of cases occurred in the first week of calving in the unsupplemented cows. Cases of white line lesion did not decline significantly until after week 23. Peaks of white line lameness were mainly observed in weeks 0-4, 7-14 and 17-23.

Figure 3.14: White line lesion cases by trial month and supplementation

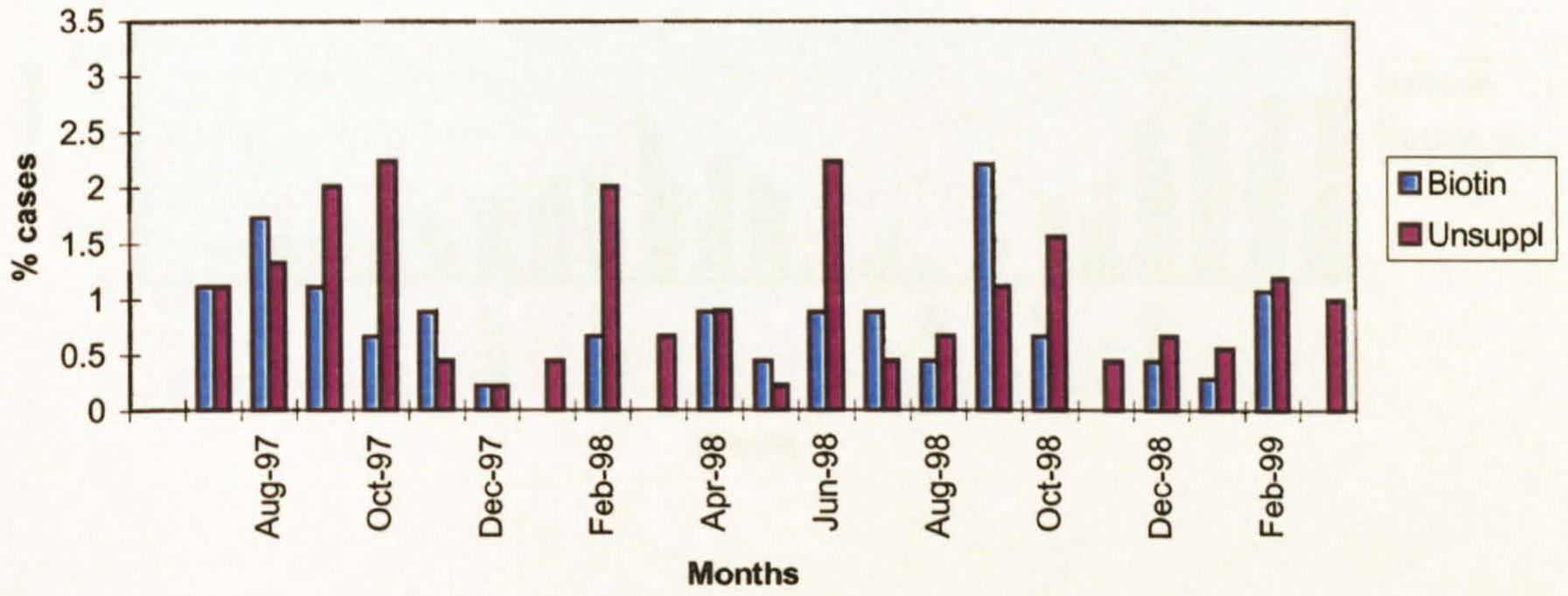
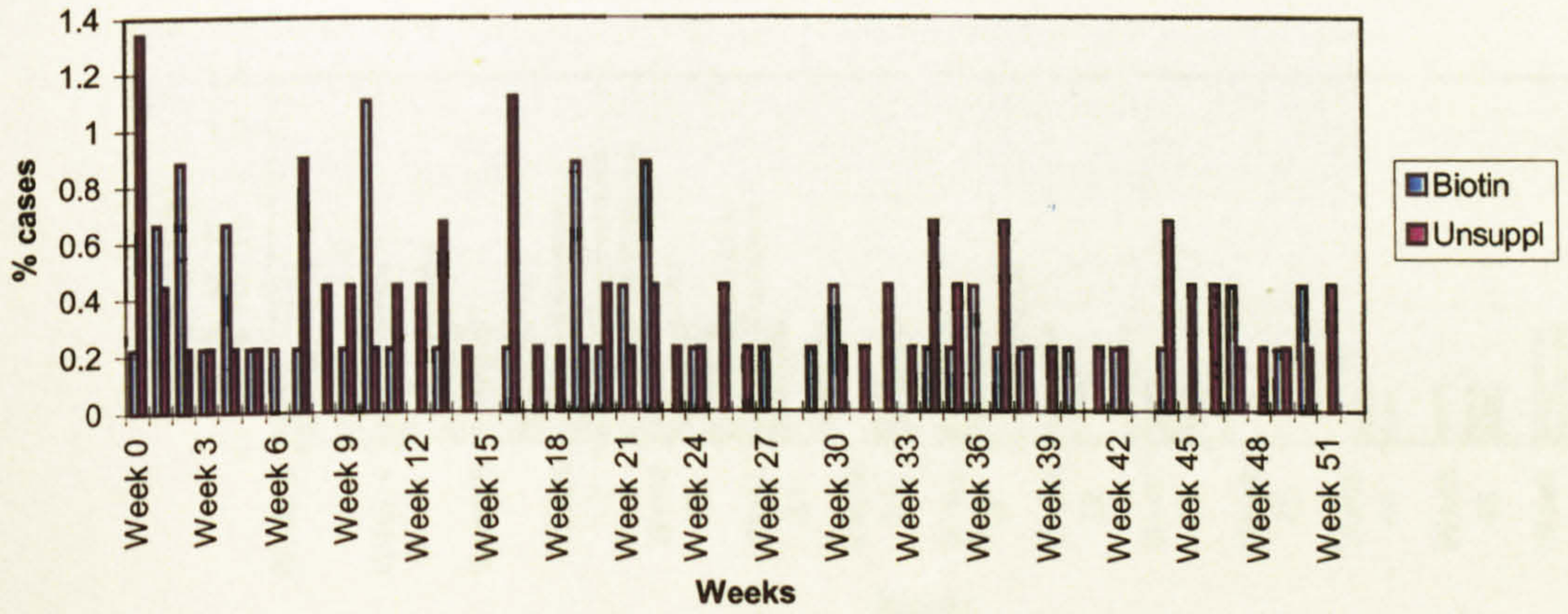


Figure 3.15: Pattern of white line lesion lameness after calving



Digital dermatitis followed a definite seasonal pattern which applied to both biotin supplemented and unsupplemented animals (Figure 3.16). This lesion occurred most predominantly during the winter housing months October 1997 to May 1998 and October 1998 to the end of the trial. It is evident that the proportion of digital dermatitis cases was significantly lower during the months of June, July, August and September. Digital dermatitis occurred mostly in weeks 11-19 after calving (Figure 3.17).

Figure 3.16: Digital dermatitis cases by trial month and supplementation

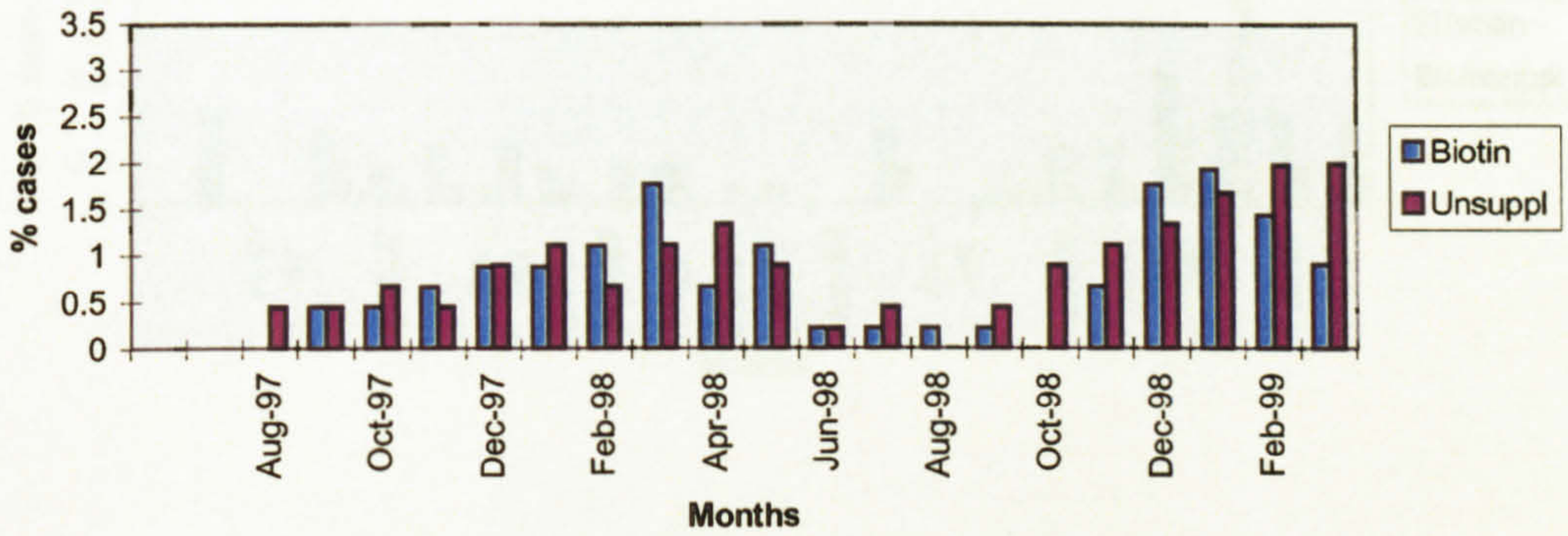
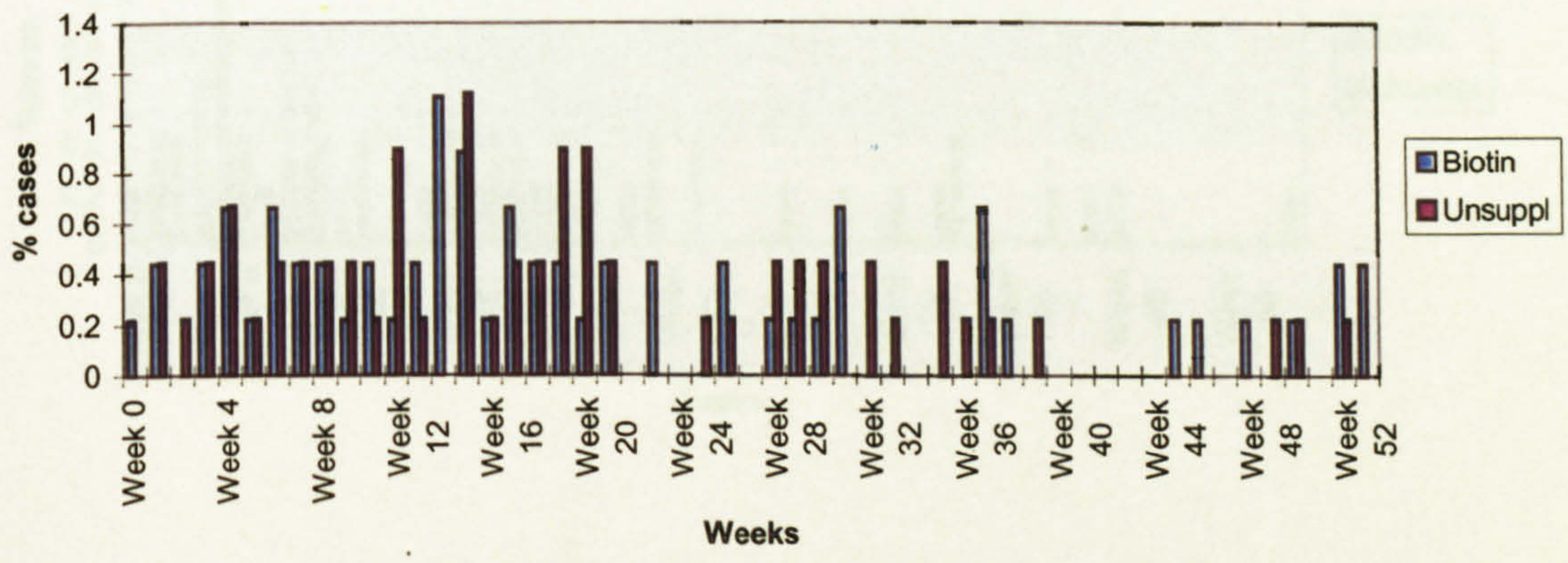


Figure 3.17: Pattern of digital dermatitis after calving



A similar seasonal pattern with interdigital necrobacillosis was observed (Figure 3.18). The majority of the cases of interdigital necrobacillosis occurred during the winter housing months 1998/99 and particularly the unsupplemented cows in January 1999, although there was a sudden increase in July 1998. The majority of cases occurred 0-8 weeks after calving and very few cases occurring after 23 weeks (Figure 3.19).

Figure 3.18: Interdigital necrobacillosis cases by trial month and supplementation

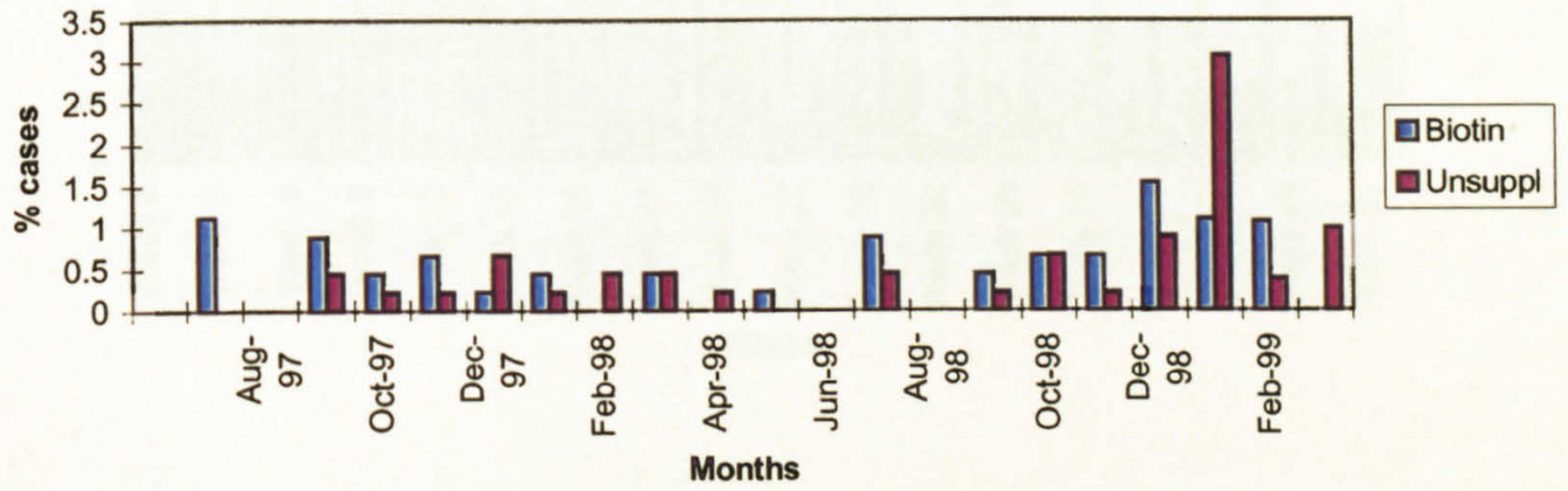
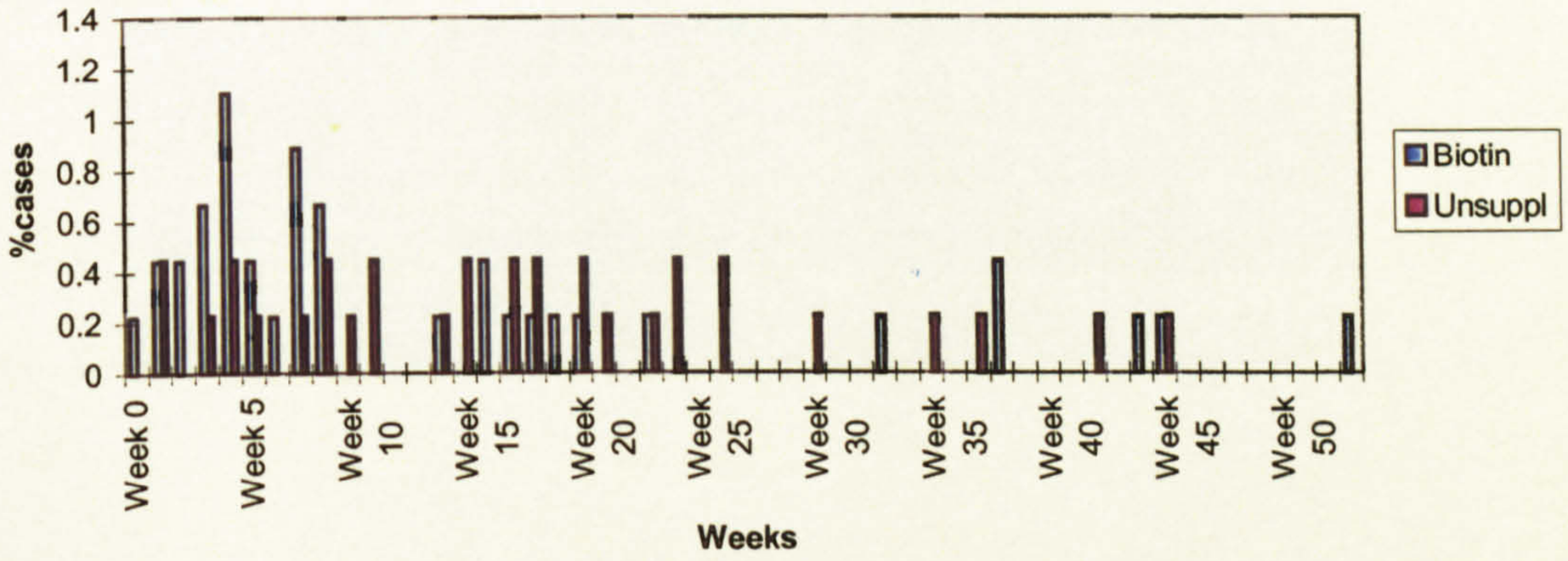
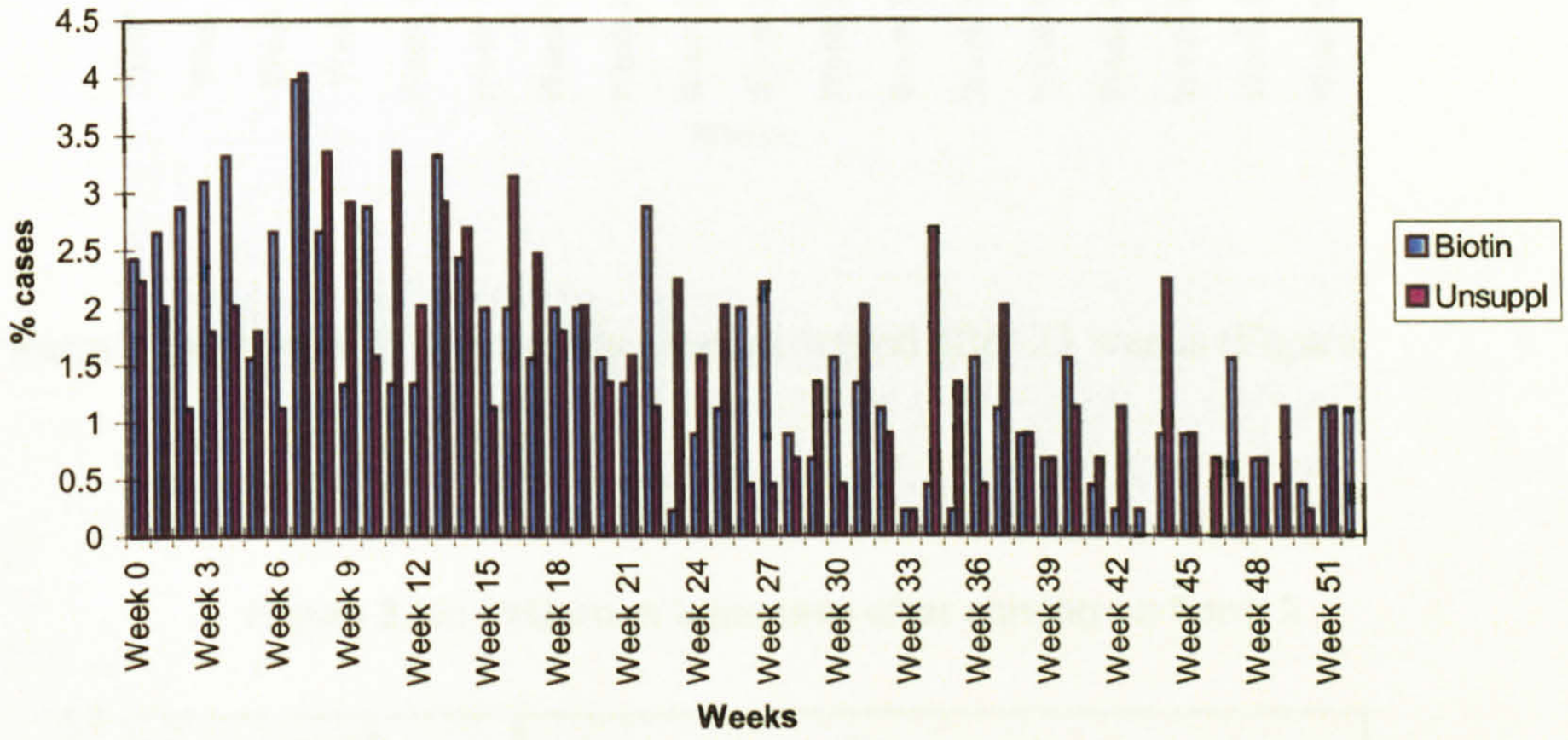


Figure 3.19: Pattern of interdigital necrobacillosis after calving



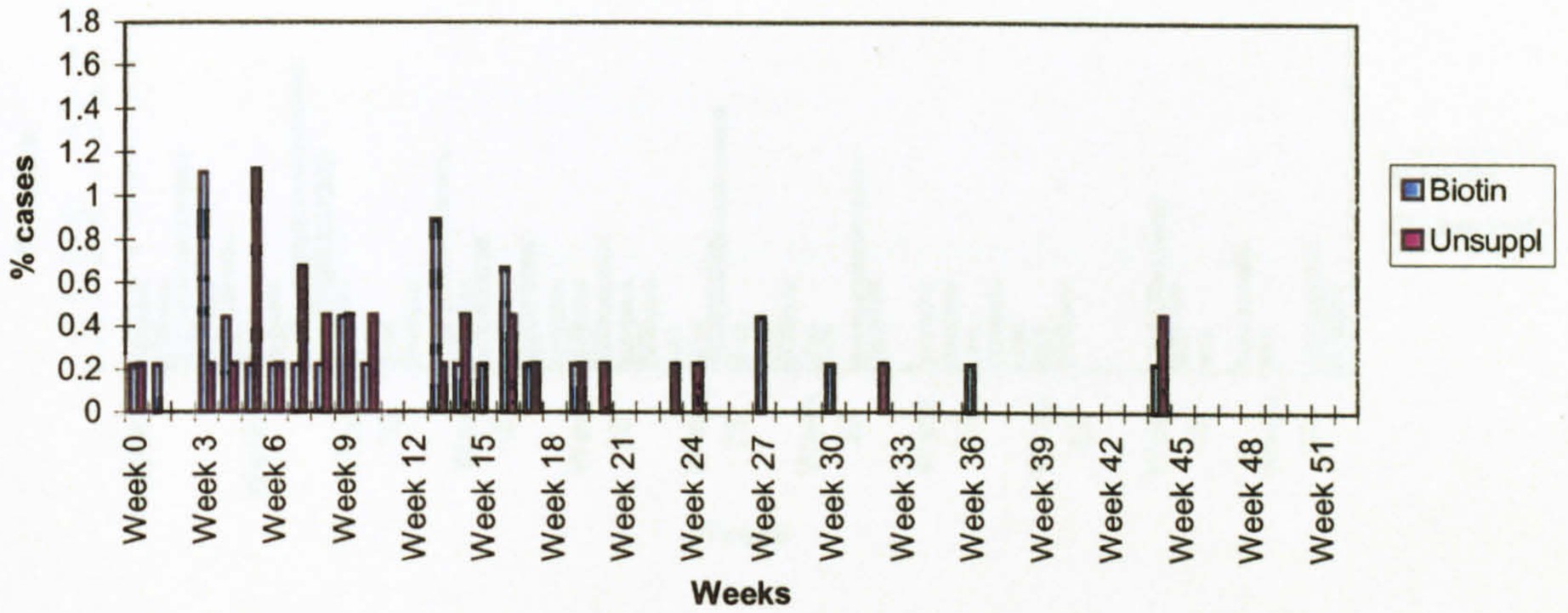
The pattern of all lameness was plotted from calving. Lameness started to rise immediately following calving to peak at week 7 and decline gradually until approximately week 27 after which lameness occurrence fluctuated (Figure 3.20).

Figure 3.20: Pattern of lameness occurrence after calving (all farms)



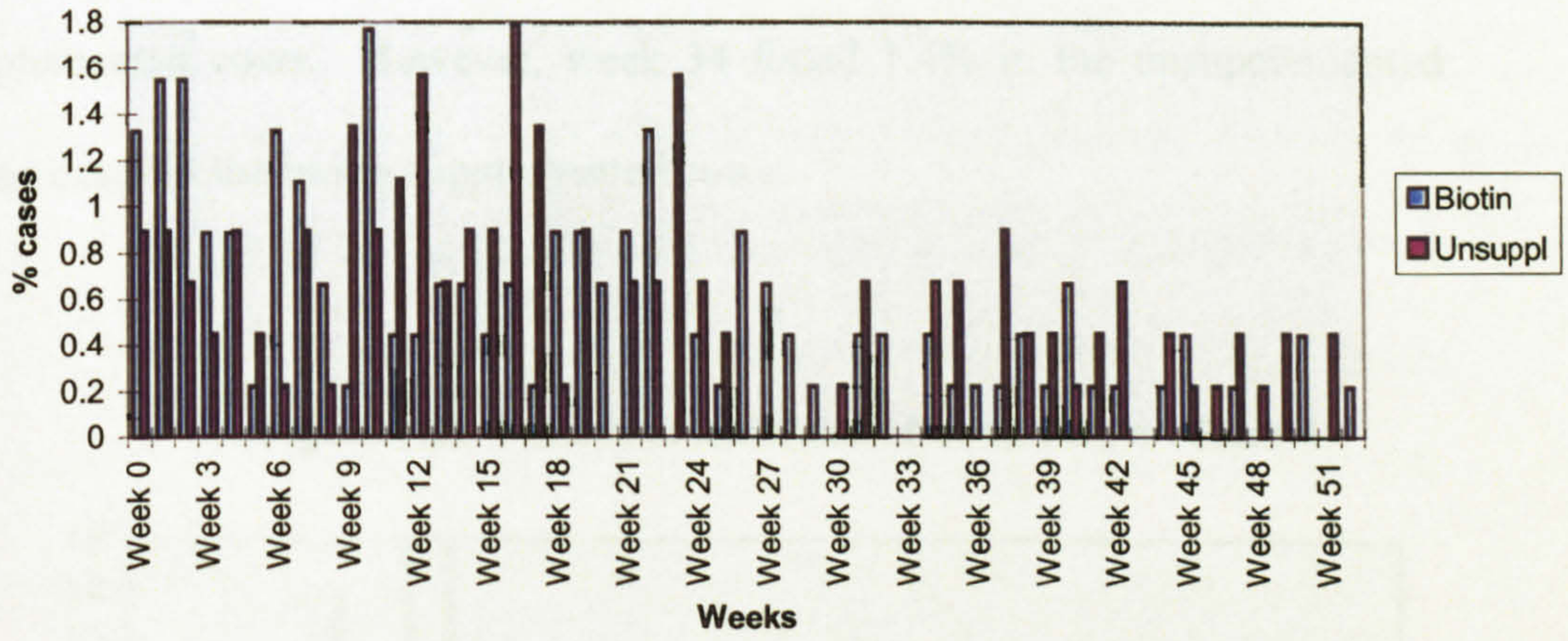
The pattern of lameness after calving was plotted by Farm. Farm 1 had a low incidence of lameness, the majority of which occurred between weeks 3 and 10 following calving (Figure 3.21).

Figure 3.21: Pattern of lameness after calving on Farm 1



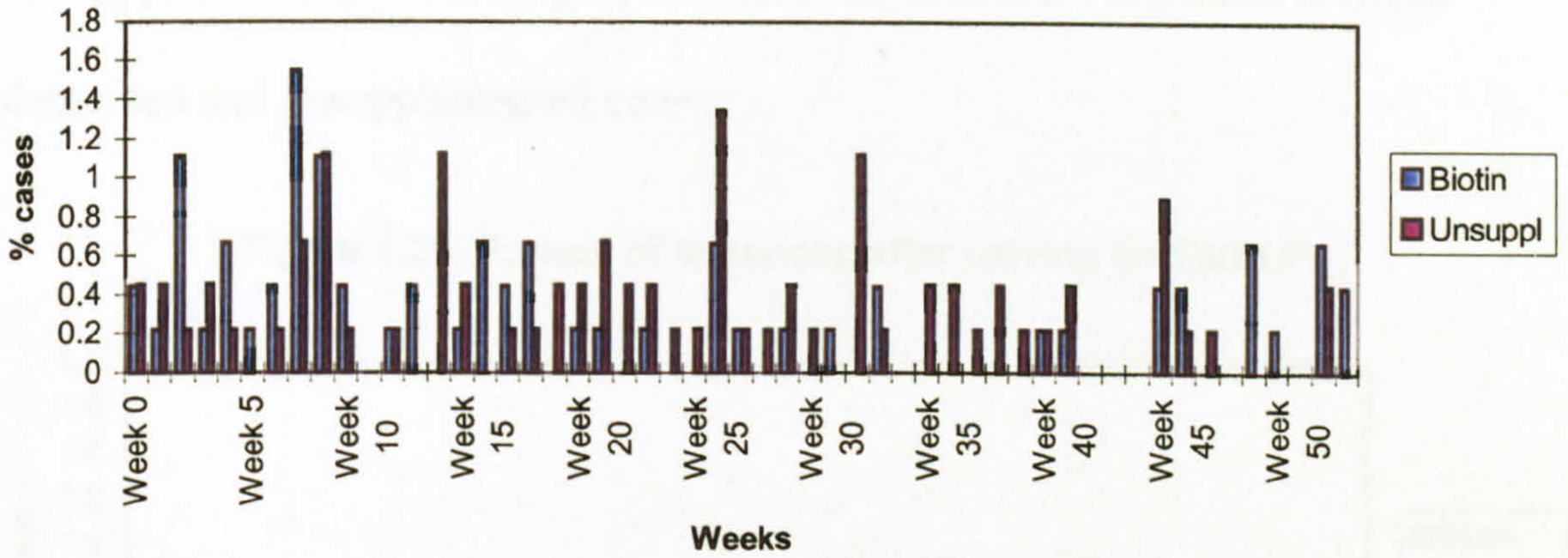
On Farm 2 there were few lameness cases observed after 23 weeks (Figure 3.22).

Figure 3.22: Pattern of lameness after calving on Farm 2



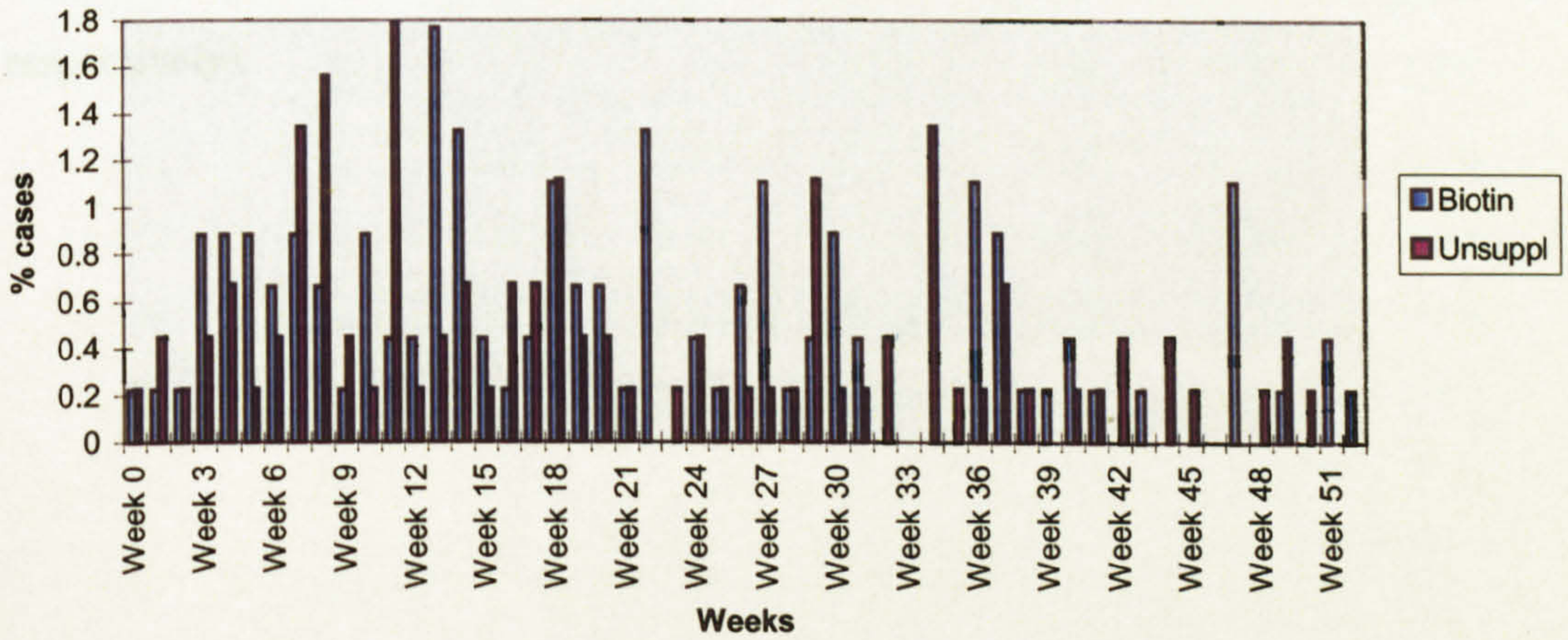
There was no clear pattern in incidence of lameness after calving on Farm 3 (Figure 3.23), although the majority of lameness appeared to occur closer to calving.

Figure 3.23: Pattern of lameness after calving on Farm 3



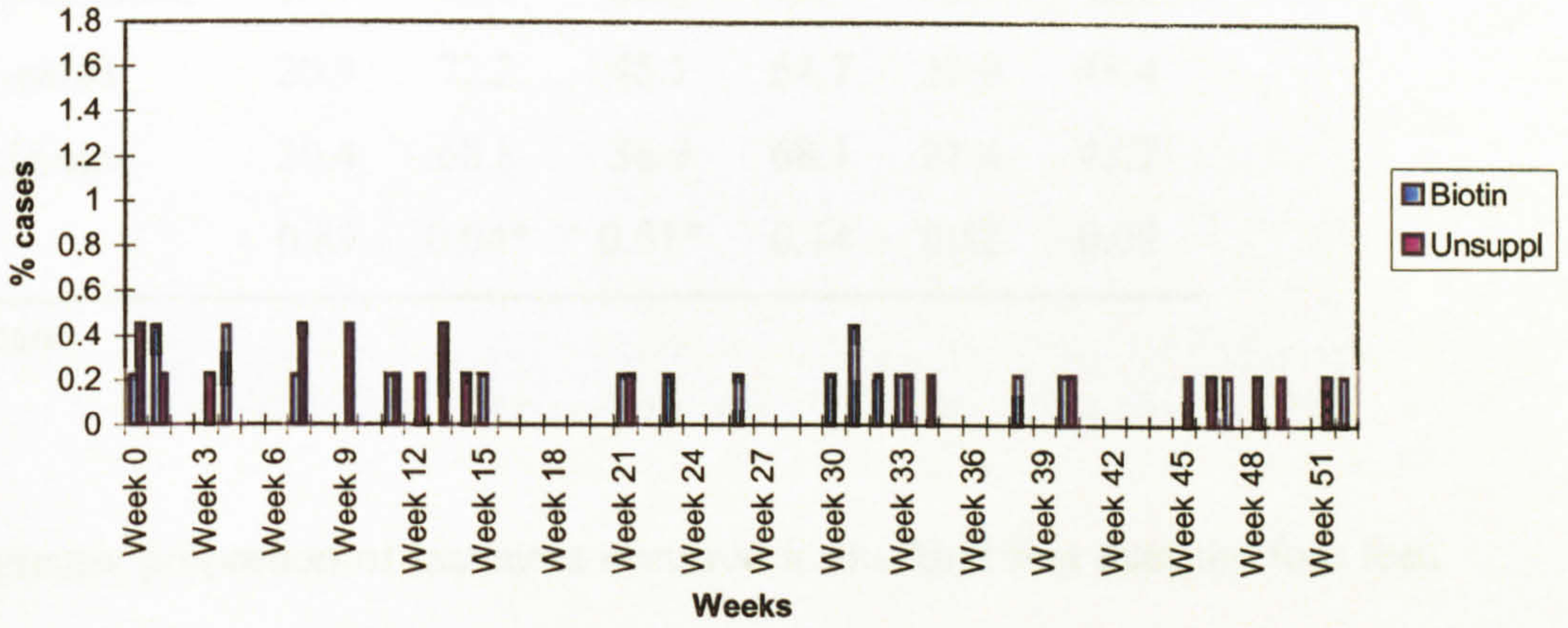
The majority of lameness after calving on Farm 4 (Figure 3.24) occurred around weeks 7, 8, 11, 13 and 14. Lameness in the biotin supplemented cows was approximately 1.4% in week 22 and 1.2 % cases in week 47, and no cases occurred in the unsupplemented cows. However, week 34 found 1.4% in the unsupplemented cows and no cases in the biotin supplemented cows.

Figure 3.24: Pattern of lameness after calving on Farm 4



Farm 5 had very few cases per week of lameness following calving. The maximum was approximately 0.4% (Figure 3.25) and no difference appeared between biotin supplemented and unsupplemented cows.

Figure 3.25: Pattern of lameness after calving on Farm 5



The incidence rate of the four most common causes of lameness, sole ulcer, white line lesion, digital dermatitis and interdigital necrobacillosis were compared (Table 3.24). The unsupplemented cows had a greater incidence of lameness as a result of the four lesions when compared to the biotin supplemented cows on all farms except for Farm 4 and a significant difference was observed on Farm 2 and 3 (P 0.04 and P0.01 respectively).

Table 3.24: Incidence rate per 100 cows per year of the four most common causes of lameness combined; sole ulcer, white line lesion, digital dermatitis and interdigital necrobacillosis, by farm and biotin supplementation

Farm ID	1	2	3	4	5	Total
Biotin Supplemented	19.7	65.6	28.8	70.7	13.7	43.0
Unsupplemented	20.9	72.2	45.1	64.7	20.9	48.4
Overall incidence	20.4	68.8	36.9	68.1	21.4	45.7
P	0.89	0.04*	0.01*	0.14	0.42	0.09

*P Significant.

A greater proportion of lameness occurred in the hind feet than the fore feet. The lateral claw of the hind feet had a higher incidence (13.2 left, 15.3 right) than the medial claws (3.6 left and right) (Table 3.25) and the difference remained on all five farms.

Table 3.25: Overall lameness incidence by limb and claw by farm

Feet	LF		RF		LH		RH	
FARMS	Lateral	Medial	Lateral	Medial	Lateral	Medial	Lateral	Medial
1	0	1.45	0	0	2.90	0.48	7.26	1.93
2	2.46	6.97	2.05	6.56	32.40	8.61	32.40	7.79
3	0.74	2.21	1.47	2.58	10.31	3.68	12.88	3.31
4	0.76	4.19	0.38	5.33	9.52	2.28	11.04	2.66
5	0.74	0.74	1.48	1.48	7.39	1.48	10.35	0.74
Total	0.98	3.39	1.07	3.48	13.21	3.57	15.36	3.57

LF - Left fore, LH - Left hind, RF - Right fore, RH - Right hind.

Repeat lameness examinations

No lesion occurred twice in the same location in the foot within one lactation throughout the duration of the trial. Repeat visits were defined as examinations of a previously diagnosed and treated lesion that was still a problem or causing lameness in a given lactation, any lesions situated in different areas on the same claw as this site or other claws were treated as new lesions.

Appendix IV details the repeat visits to secondary lesions. The overall incidence of these visits were low, the highest incidence observed in a single lesion was under run sole 0.6 per 100 cows per year. No significant differences were observed between the biotin supplemented and unsupplemented cows at such a low incidence level.

Farm 2 had the highest incidence of repeat visits by the veterinarians (Table 3.26). Biotin supplemented cows had an incidence of 15.6 which was significantly fewer repeat visits than the incidence observed in the unsupplemented cows 27.9 (P 0.02). The overall incidence of farm pooled repeat examinations also identified a greater incidence in the unsupplemented cows (15.2) than the biotin supplemented cows (11.1) but the difference was marginally significant P = 0.06. No significant differences were observed between the biotin supplemented and unsupplemented cows on the other four farms.

Table 3.26: Incidence of repeat examinations by supplementation and farm

	FARMS					
Biotin	1	2	3	4	5	Total
Yes	0.99	15.58	11.53	19.55	1.37	11.09
No	0.95	27.91	18.05	16.96	4.83	15.21
Total	0.97	21.74	14.72	18.27	2.96	13.12
P	1.00[a]	0.02*	0.23	0.65	0.34[a]	0.06

*P Significant (Yates corrected, [a] 2-tailed fisher exact)

The repeat examinations were analysed by the four most common lesions. No repeat examinations for sole ulcer lesions were reported on Farm 1 and in the biotin supplemented cows on Farm 5 or the unsupplemented cows on Farm 3 (Table 3.27). Again the farm with the highest incidence of repeat examinations was Farm 2. Unsupplemented cows had a greater incidence of repeat examinations (9.0) than the biotin supplemented cows (8.2) but the difference was not significant. No significant difference was observed between the biotin supplemented and unsupplemented cows in the repeat examinations of sole ulcer lesions.

Table 3.27: Incidence of repeat examinations of sole ulcer by supplementation and farm

		FARMS					
	Biotin	1	2	3	4	5	Total
Sole ulcer	Yes	0	8.20	0.72	1.50	0	2.29
	No	0	9.03	0	0.77	3.22	2.53
	Total	0	8.61	0.37	1.14	2.96	2.41
	P value	0	1.00	0.50[a]	1.00[a]	0.22[a]	0.97

*P Significant (Yates corrected, [a] 2-tailed fisher exact)

Repeat examination incidence of white line lesion lameness were significantly different by supplementation overall. Biotin supplemented cows were re-examined less than the unsupplemented cows (Table 3.28). Farm 2 had the greatest incidence of repeat examinations of white line lesion lameness and the unsupplemented cows (12.3) had a greater incidence than biotin supplemented cows (4.1) P = 0.03. Biotin supplemented (1.4) cows observed on Farm 3 also had significantly fewer (P 0.04) repeat examinations of white line lesion lameness than the unsupplemented cows (7.52). Farm 1 and 5 did not have any white line lesion lameness repeat examinations.

Table 3.28: Incidence of repeat examinations of white line lesion by supplementation and farm

		FARMS					
	Biotin	1	2	3	4	5	Total
White Line	Yes	0	4.10	1.44	2.26	0	1.76
	No	0	12.31	7.52	0.77	0	4.71
	Total	0	8.20	4.42	1.52	0	3.21
	P value	0	0.033*	0.039*	0.62	0	0.010*

*P Significant (Yates corrected, [a] 2-tailed fisher exact)

Farm 2 and 5 and the biotin supplemented cows on Farm 1 did not receive any repeat examinations for digital dermatitis throughout the trial (Table 3.29). Like the incidence figures for primary lesions, Farm 4 had the highest repeat incidence for digital dermatitis 12.8 biotin supplemented cows and 10.8 in the unsupplemented cows. The biotin supplemented cows also received a greater incidence of repeat

examinations in two of the remaining three farms although no significant differences were observed.

Table 3.29: Incidence of repeat examinations of digital dermatitis by supplementation and farm

		FARMS					
	Biotin	1	2	3	4	5	Total
Digital Derm.	Yes	0	0	5.05	12.79	0	4.22
	No	0.95	0	3.76	10.79	0	3.62
	Total	0.48	0	4.42	11.80	0	3.93
	P value	1.00[a]	0	0.75	0.72	0	0.67

*P Significant (Yates corrected, [a] 2-tailed fisher exact)

Repeat examinations for interdigital necrobacillosis only occurred on two farms (Table 3.30). The greatest incidence occurred on Farm 4 in the unsupplemented cows 4.6 and 1.5 in the biotin supplemented cows. The unsupplemented cows had a higher incidence than the biotin supplemented cows on Farm 2 but, incidence was quite low, therefore no significance was observed between supplementation.

Overall, white line lesion lameness was identified as the lesion that was most significantly affected by biotin supplementation. The figures shown in Table 3.31 display a significant reduction in white line lameness on Farm 3 (P 0.04) and when all data were pooled from the five farms (P 0.01). These data and the data for sole ulcer, digital dermatitis and interdigital necrobacillosis were further analysed using multivariate techniques to identify any effects imposed by independent variables. The other lesions identified in the trial were not analysed further as too few cases were identified to create viable findings.

Table 3.30: Incidence of repeat examinations of interdigital necrobacillosis by supplementation and farm

		FARMS					
	Biotin	1	2	3	4	5	Total
Interdig. necro	Yes	0	0.82	0	1.50	0	0.53
	No	0	1.64	0	4.62	0	1.45
	Total	0	1.23	0	3.04	0	0.98
	P value	0	1.00[a]	0	0.17[a]	0	0.22

*P Significant (Yates corrected, [a] 2-tailed fisher exact)

Table 3.31: Incidence of white line lesion lameness by farm and supplementation

Farm ID	1	2	3	4	5	Total
Biotin Supplemented	2.0	24.6	7.9	7.5	5.5	10.0
Unsupplemented	3.8	33.6	17.3	7.7	11.3	15.4
Total	2.9	29.1	12.5	7.6	8.1	12.7
P	0.68	0.13	0.04*	0.83	0.39	0.01*

**P* significant.

Multivariate analysis

Data for the four most common lesions were analysed using Cox proportional hazard survival analysis.

An entry time variable was used for the time difference (days) between the day the first cow on the first farm started the trial and the day each individual cow started the trial after this, on all five farms. Time to event was estimated from day one of entry to the day of the occurrence of a specific lesion to identify the hazard for an

exposure. Any cows that did not become lame with the specific lesion were coded as right censored (not failing or having the lesion in the given time frame) (Cox 1972). This did not necessarily mean that the cows did not develop this lesion after the trial finished.

Four separate models were used to test the time to failure of sole ulcer, white line lesion, digital dermatitis and interdigital necrobacillosis. Farm, as a fixed effect, was forced into each of these models to test the within herd design of the trial and biotin supplementation was also included. Other independent variables were also tested in the models and interactions were investigated.

Sole ulcer was tested using the Cox proportional survival model (Table 3.32). There was a significant difference in the incidence of sole ulcer on Farms 2 and 4 when compared to the baseline of Farm 1. Biotin supplementation was not significant hazard ratio (HR) 1.05 (confidence interval (CI) 0.76 to 1.44) (P = 0.77).

Digital dermatitis data (Table 3.33) were analysed using a similar Cox proportional survival analysis model and no difference was observed between biotin supplemented and unsupplemented animals P = 0.58 (HR 0.91 and CI 0.64 to 0.58) . Farm 5 did not have any digital dermatitis so it was not included in this analysis. Farm 1 was the baseline and Farm 3 and 4 were significantly different from Farm1 (P 0.001).

Table 3.32: Cox proportional hazard survival analysis of sole ulcer incidence by farm and biotin supplementation

Variable	level	No. cattle	Coefficient	Standard error	hazard ratio	lower CI	Upper CI	*P*
Biotin supplemented	Yes	453	0.05	0.16	1.05	0.76	1.44	0.77
Farm	2	180	2.21	0.37	9.12	4.40	18.90	<0.001*
	3	227	0.52	0.42	1.67	0.73	3.84	0.22
	4	215	1.28	0.39	3.61	1.68	7.76	<0.001*
	5	111	0.56	0.47	1.76	0.69	4.46	0.24

* *P* significant, CI = 95% confidence interval

Table 3.33: Cox proportional hazard survival analysis of digital dermatitis incidence by farm and biotin supplementation

Variable	level	No. cattle	Coefficient	Standard error	hazard ratio	lower CI	Upper CI	*P*
Biotin supplemented	Yes	453	-0.09	0.17	0.91	0.64	1.28	0.58
Farm	2	180	0.15	0.61	1.16	0.35	3.82	0.80
	3	227	1.65	0.48	5.19	2.03	13.27	<0.001*
	4	215	2.57	0.46	13.02	5.27	32.18	<0.001*

* *P* significant, CI = 95% confidence interval

Interdigital necrobacillosis had a HR 1.06 which was not significant P = 0.22 (CI 0.68 to 1.66). Therefore biotin supplementation did not reduce the incidence of this lesion (Table 3.34). Farm 3 and 5 were significantly different to the baseline Farm 1.

Table 3.34: Cox proportional hazard survival analysis of interdigital necrobacillosis lameness by farm and biotin supplementation

Variable	level	No. cattle	Coefficient	Standard error	hazard ratio	lower CI	Upper CI	*P*
Biotin supplemented	Yes	453	0.06	0.22	1.06	0.68	1.66	0.22
Farm	2	180	-0.49	0.35	0.61	0.31	1.22	0.16
	3	227	-1.21	0.38	0.30	0.14	0.63	0.002*
	4	215	-0.02	0.28	0.98	0.56	1.70	0.93
	5	111	-2.81	1.02	0.06	0.01	0.45	0.006*

* *P* significant, CI = 95% confidence interval

Table 3.35 shows the significant effect of biotin supplementation on the hazard of white line lesion. HR 0.59 meant a significant reduction of white line lesion (P 0.002, CI 0.42 to 0.83) in cows supplemented with biotin. There was an increasing hazard for white line lesion with increasing lactation number. Lactation 2 was not significantly different to the baseline of first lactation, but by lactation 3 HR = 2.66, P 0.03 a significant difference was observed when compared to lactation 1 and the confidence interval became broader with each lactation. The final lactation confidence interval was very broad as it incorporated a few larger lactation numbers up to 12 lactations.

Table 3.35: Cox proportional hazard survival analysis of white line lesion lameness incidence by biotin and lactation number

Variable	level	No. cattle	Coefficient	Standard error	hazard ratio	lower CI	Upper CI	P
Biotin	Yes	453	-0.53	0.17	0.59	0.42	0.83	0.002*
Lactation at	2	200	0.82	0.45	2.28	0.95	5.48	0.07
trial end	3	165	0.98	0.45	2.66	1.10	6.46	0.03*
	4	123	1.69	0.44	5.40	2.30	12.70	<0.001*
	5	89	1.75	0.44	5.78	2.44	13.65	<0.001*
	6	42	2.14	0.47	8.50	3.34	21.59	<0.001*
	≥7	49	3.01	0.43	20.31	8.72	47.29	<0.001*

*P significant, CI = 95% confidence interval

As a result of the unreliable supplementation encountered on Farm 1 these data were removed from the analysis to detect any significant alteration in the model findings (Table 3.36). There was a significant effect of biotin supplementation on white line lameness HR 0.59, P 0.004, CI 0.42 to 0.84 and the significance of lactation remained beyond lactation 4 (P <0.001). Farms 3, 4 and 5 were significantly different to the baseline Farm 2 (P <0.001), with narrow CI all below 1.

Table 3.36: Cox proportional hazard survival analysis of white line lesion lameness incidence by lactation number and biotin supplementation, excluding Farm one

Variable	level	No. cattle	Coefficient	Standard error	hazard ratio	lower CI	upper CI	P
Biotin	Yes	453	-0.52	0.18	0.59	0.42	0.84	0.004*
Lactation at	2	200	0.87	0.45	2.38	0.99	5.72	0.05*
trial end	3	165	0.86	0.46	2.36	0.95	5.86	0.06
	4	123	1.71	0.44	5.54	2.34	13.08	<0.001*
	5	89	1.74	0.44	5.75	2.43	13.59	<0.001*
	6	42	2.14	0.47	8.49	3.34	21.58	<0.001*
	≥7	49	2.96	0.43	19.30	8.25	45.11	<0.001*
Farm	Three	227	-0.82	0.22	0.44	0.29	0.68	<0.001*
	Four	215	-1.16	0.27	0.31	0.18	0.53	<0.001*
	Five	111	-1.67	0.34	0.19	0.10	0.37	<0.001*

* P significant, CI = 95% confidence interval

The effect of removing Farm 3 was also investigated because the biotin milk levels were not significantly different between supplements in individual animals. The Cox proportional hazard survival analysis model was applied to the white line data from Farms 2, 4 and 5 only (Table 3.37). The effect of biotin on white line lesion lameness became marginal (P 0.07), HR 0.69 and CI 0.46 to 1.03. However, the removal of both Farm 1 and 3 from the analysis also compromised the power of the trial and analysis. Farm 1 and 3 contributed a total of 394 (44%) of the cows involved in the study. Farm 3 alone was the largest trial farm, 227 cows.

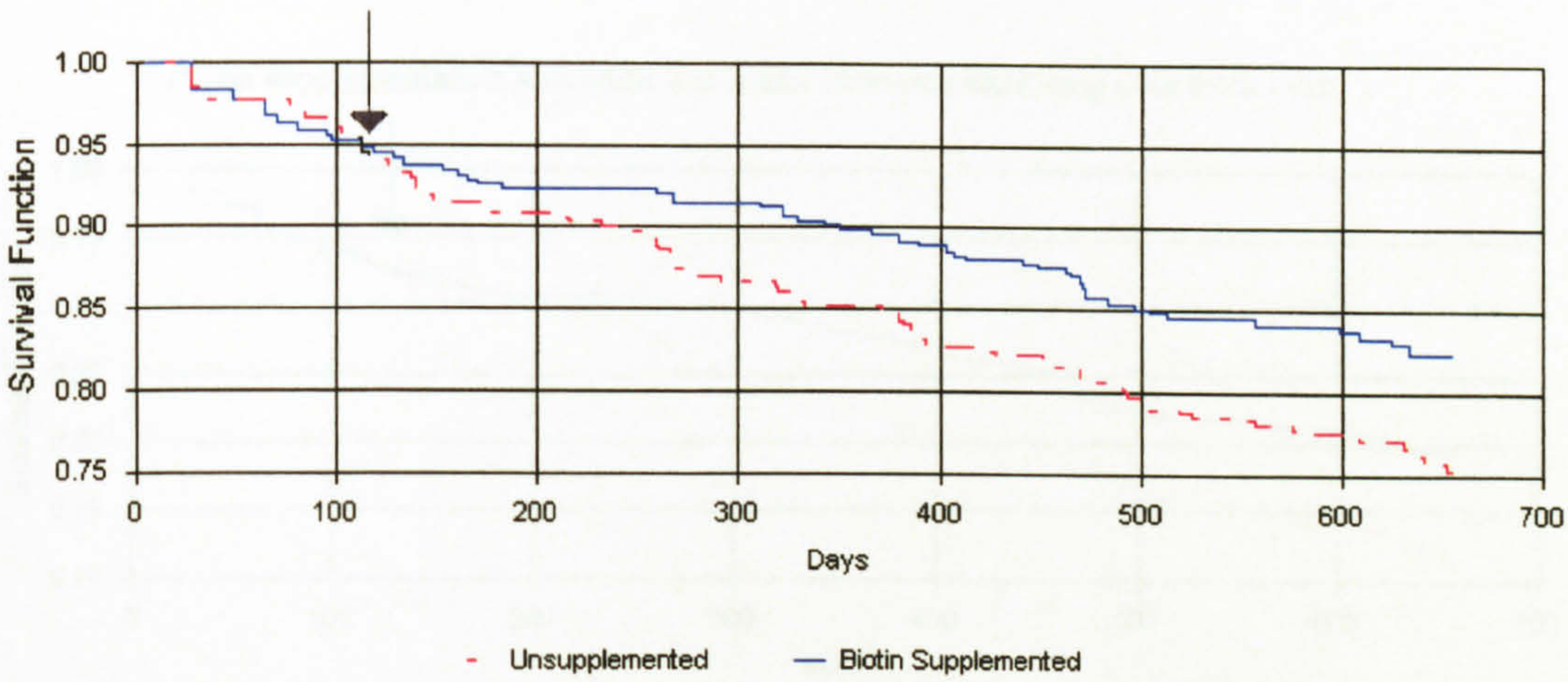
Table 3.37: Cox proportional hazard survival analysis of white line lesion lameness incidence by lactation number and biotin supplementation, excluding Farm one and three

Variable	level	No. cattle	Coefficient	Standard error	hazard ratio	lower CI	upper CI	P
Biotin	Yes	453	-0.37	0.20	0.69	0.46	1.03	0.07
Lactation at trial end	2	200	0.83	0.48	2.30	0.90	5.86	0.08
	3	165	0.74	0.51	2.10	0.76	5.76	0.15
	4	123	1.54	0.48	4.68	1.83	11.94	0.001*
	5	89	1.57	0.48	4.83	1.87	12.45	0.001*
	6	42	1.92	0.52	6.81	2.45	18.98	<0.001*
	≥7	49	2.42	0.48	11.22	4.35	28.96	<0.001*
Farm	Four	215	-1.31	0.28	0.27	0.15	0.46	<0.001
	Five	111	-1.69	0.35	0.18	0.09	0.36	<0.001

* P significant, CI = 95% confidence interval

The survival function for white line was plotted using a Kaplan Meier curve (Hosmer and Lemeshow 1999). This displayed the probability of survival by the proportion alive or not having white line lesion lameness on any given day given that they had not failed previously. The plot begins with 1 or 100% or the population alive or not failed to white line lesion. With each white line lesion lameness case the plot steps down from 1 (Altman 1991). Figure 3.26 shows a Kaplan Meier curve plot which included the analysis of all five farms. Unsupplemented cows experienced greater incidence of white line lesion and the difference is clearly observed in Figure 3.26 where the line falls considerably in the unsupplemented cows. The divergence between biotin supplemented cows and unsupplemented cows appears in this plot at approximately 130 days.

Figure 3.26: Survival function for Kaplan-Meier estimation



To confirm that Farm 1 did not have a significant effect on the difference between biotin supplemented and unsupplemented cows with white line lesion lameness a Kaplan Meier curve was plotted omitting the data from Farm 1 (Figure 3.27). The plot is very similar to the plot for all five farms. Divergence still occurs at approximately 130 days.

Due to the divergence of white line lesion lameness observed between biotin supplemented and unsupplemented cows at approximately 130 days, data were analysed using the Cox proportional hazard survival analysis with the omission of the first 130 days of data from each animal (Table 3.38). Removal of this data provided a HR 0.54, P 0.003 and CI 0.35 to 0.81, a reduced hazard ratio than that observed with the complete data set (HR 0.59)

A Kaplan Meier curve plotting the data following the removal of the first 130 days confirmed that the divergence of hazard between the biotin supplemented and unsupplemented cows occurred at 130 days (Figure 3.28).

Figure 3.27 : Survival function for Kaplan-Meier estimation

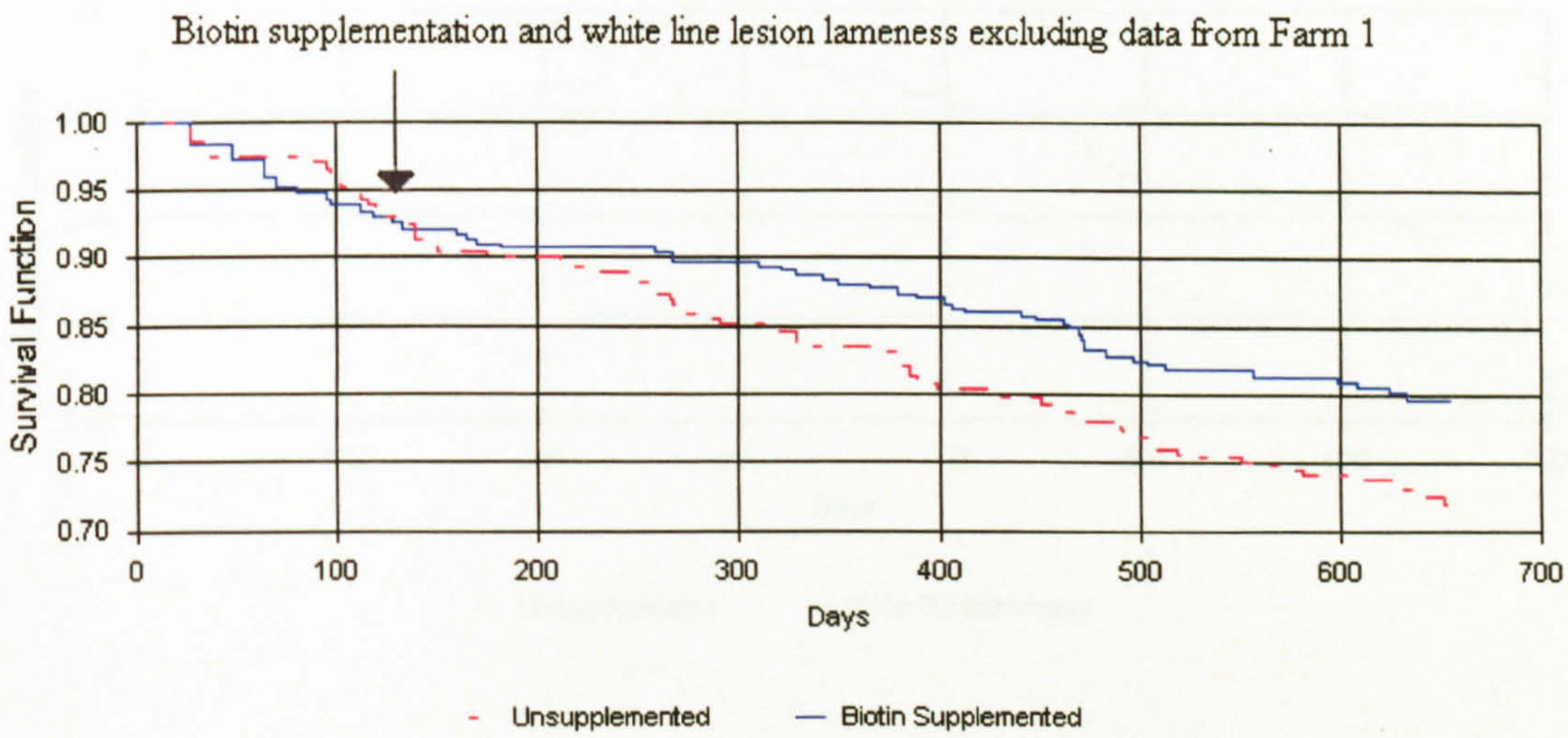
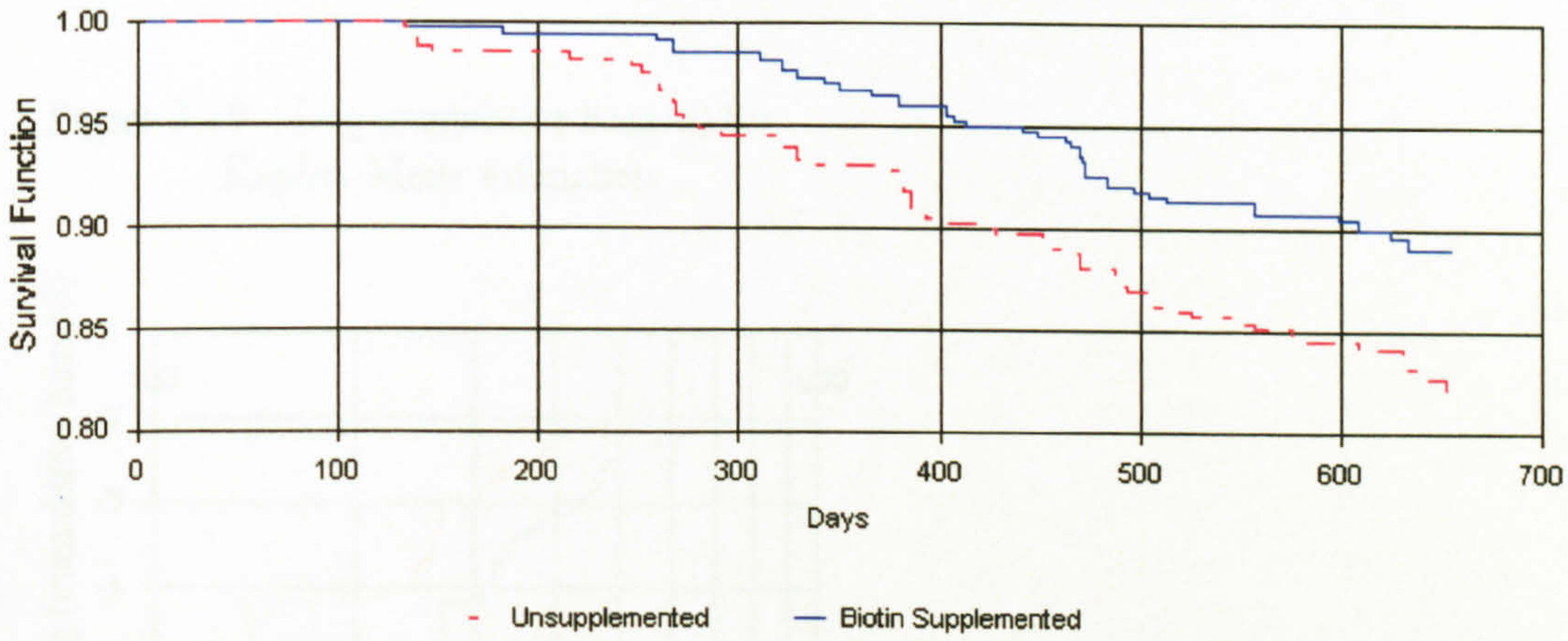


Table 3.38: Cox proportional hazard survival analysis of white line lesion lameness incidence by lactation number and biotin supplementation after the removal of the first 130 days of data

Variable	level	No. cattle	Coefficient	Standard error	hazard ratio	lower CI	Upper CI	P
Biotin	Yes	453	-0.62	0.21	0.54	0.35	0.81	0.003*
Lactation at	2	200	0.76	0.50	2.15	0.81	5.70	0.12
trial end	3	165	0.94	0.50	2.56	0.95	6.89	0.06
	4	123	1.63	0.49	5.12	1.97	13.30	<0.001*
	5	89	1.90	0.47	6.70	2.64	16.99	<0.001*
	6	42	2.23	0.52	9.32	3.35	25.94	<0.001*
	≥7	49	3.05	0.48	21.18	8.33	53.83	<0.001*

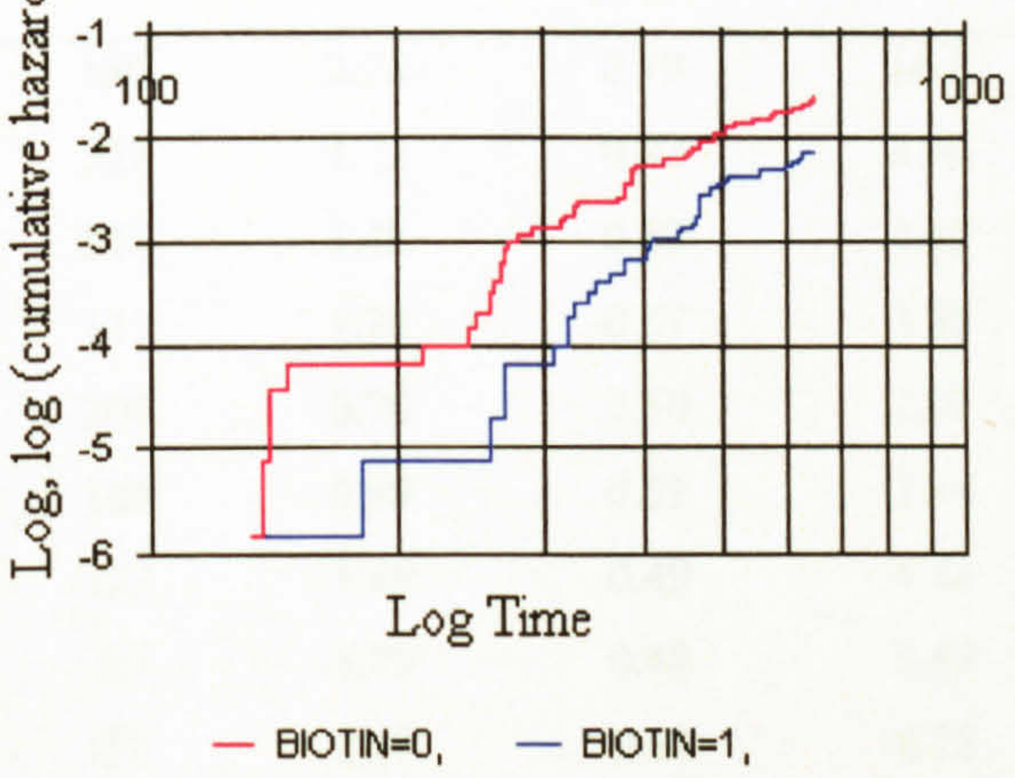
* *P* significant, CI = 95% confidence interval

Figure 3.28 : Survival function for Kaplan-Meier estimation



Analysis of the goodness of fit of the model (Figure 3.29) using the log cumulative hazard for Kaplan Meier estimation confirmed that the model used in this analysis was an appropriate fit. At no point in the plot did biotin supplemented and unsupplemented lines converge or cross.

Figure 3.29 : Log (cumulative hazard) for
Kaplan-Meier estimation



0 = Unsupplemented

1 = Biotin supplemented

There were no significant interactions. The effect of biotin was therefore, considered to be consistent across farms and lactations (Table 3.39-3.41).

Table 3.39: Farm tested for interactions within the model (minus the first 130 days)

(baseline not supplemented)

Variable	No. cattle	Coefficient	Standard error	hazard ratio	lower CI	Upper CI	P
Farm 2	180	2.78	0.79	16.1	3.42	75.70	<0.001*
Farm 3	227	1.32	0.82	3.76	0.75	18.82	0.11
Farm 4	215	1.48	0.82	4.41	0.87	22.26	0.07
Farm 5	111	1.22	0.87	3.39	0.61	18.70	0.16
Lact 2	200	0.70	0.50	2.01	0.75	5.35	0.16
Lact 3	165	0.89	0.51	2.44	0.91	6.59	0.08
Lact 4	123	1.49	0.49	4.44	1.69	11.64	0.002*
Lact 5	89	1.79	0.48	5.97	2.32	15.34	<0.001*
Lact 6	42	2.19	0.52	8.98	3.22	25.06	<0.001*
Lact 7+	49	2.94	0.48	18.96	7.41	48.48	<0.001*
Biotin	453	-0.68	0.21	0.51	0.33	0.77	0.001*
Farm 1 Biotin	167	-0.82	1.10	0.44	0.05	3.75	0.45
Farm 2 Biotin	180	-0.50	0.28	0.61	0.35	1.05	0.07
Farm 3 Biotin	227	-0.11	0.36	0.89	0.44	1.82	0.75
Farm 4 Biotin	215	-0.89	0.56	0.41	0.13	1.22	0.11
Farm 5 Biotin	114	-0.38	1.08	0.69	0.08	5.70	0.73

* P significant, CI = 95% confidence interval

Table 3.40: Lactations 1 to 3 tested for interactions within the model (minus the first 130 days) (baseline not supplemented)

Variable	No. cattle	Coefficient	Standard error	hazard ratio	lower CI	Upper CI	P
Farm 2	180	2.87	0.73	17.75	4.27	73.71	<0.001*
Farm 3	227	1.55	0.75	4.74	1.09	20.64	0.04*
Farm 4	215	1.61	0.76	5.01	1.13	22.12	0.03*
Farm 5	111	1.20	0.79	3.33	0.70	15.72	0.13
Lact 2	200	0.77	0.50	2.16	0.81	5.74	0.12
Lact 3	165	0.95	0.50	2.59	0.96	6.96	0.06
Lact 4	123	1.76	0.52	5.83	2.10	16.20	<0.001*
Lact 5	89	2.02	0.50	7.51	2.81	20.08	<0.001*
Lact 6	42	2.35	0.55	10.52	3.58	30.91	<0.001*
Lact 7+	49	3.19	0.51	24.30	8.86	66.60	<0.001*
Biotin	453	-0.71	0.25	0.49	0.30	0.81	0.005*
Lact 1 to 3 biotin		0.29	0.44	1.34	0.56	3.20	0.51

* P significant, CI = 95% confidence interval

Table 3.41: Lactations 4 to 7+ tested for interactions within the model (minus the first 130 days) (baseline not supplemented)

Variable	No. cattle	Coefficient	Standard error	hazard ratio	lower CI	Upper CI	P
Farm 2	180	2.87	0.73	17.71	4.26	73.55	<0.001*
Farm 3	227	1.55	0.75	4.73	1.08	20.61	0.04*
Farm 4	215	1.61	0.76	5.00	1.13	22.08	0.03*
Farm 5	111	1.20	0.79	3.34	0.71	15.76	0.12
Lact 2	200	0.77	0.50	2.15	0.81	5.71	0.12
Lact 3	165	0.94	0.50	2.57	0.95	6.93	0.06
Lact 4	123	1.77	0.52	5.92	2.14	16.34	<0.001*
Lact 5	89	2.03	0.50	7.61	2.85	20.32	<0.001*
Lact 6	42	2.36	0.55	10.65	3.64	30.16	<0.001*
Lact 7+	49	3.20	0.51	24.65	9.05	67.18	<0.001*
Biotin	453	-0.39	0.36	0.68	0.33	1.37	0.28
Lact 4 to 7+ biotin		-0.34	0.44	0.71	0.30	1.68	0.44

* P significant, CI = 95% confidence interval

Milk data

Milk quality data were recorded each month on all trial cows and stored using a Microsoft Access database (Version 7, 1989-1995, Microsoft Corp.). These data were analysed to estimate mean values and identify any significant differences between the biotin supplemented cows and unsupplemented cows in yield, fat, lactose, protein and somatic cell count.

Table 3.42 also shows the total amount of milk in litres produced by the biotin supplemented and unsupplemented cows over the period of the trial by farm and with all farm data pooled. A significant difference was observed for total trial milk yield between supplemented and unsupplemented animals on all farms except Farm 2 and overall. However, the significantly higher yield was not consistently observed in either supplement. Farm 4 produced the highest yield 29624.1 Litres in the biotin

supplemented cows and 27425.8 Litres in the unsupplemented cows. The total yield for all cows over the entire trial was 220909.1 Litres.

A significant difference was found between the mean yield by month, in biotin supplemented and unsupplemented cows on Farm 1, 3, 4 and 5. However, Farm 1 and 4 had a higher milk yield in the biotin supplemented herd and Farm 3 and 5 had a higher yield in the unsupplemented herd. No significant difference was found on farm 2 or when all farm data were combined (Table 3.42).

A significant difference in milk fat was found between the biotin supplemented and unsupplemented cows pooled data and on Farms 3, 4 and 5 (Table 3.42). Biotin supplemented cows had milk with a lower mean fat content than the unsupplemented cows in all cases.

The mean milk lactose content was very similar in the biotin supplemented and unsupplemented cows. A significant difference was only found on Farm 2, where biotin supplemented cows had a higher mean value (Table 3.42).

The concentrations of mean milk protein content were again very similar in both biotin supplemented and unsupplemented cows except for Farm 1 and 5. Farm 1 had a greater milk protein level in the unsupplemented cows and Farm 5 greater levels were observed in the biotin supplemented cows (Table 3.42). No significant difference was observed in pooled data.

A significant difference was observed between the somatic cell counts (SCC) of biotin supplemented cows and unsupplemented cows with data pooled and on Farm 3 and 5 (Table 3.42). In all cases the biotin supplemented cows had a higher mean somatic cell count than the unsupplemented cows.

Table 3.42: Analysis of milk quality data by farm and biotin supplementation

			FARMS					
MILK DATA		2	1	2	3	4	5	All farms
Yield (Litres)	Mean	Suppl	22.81	24.50	21.29	25.74	18.34	22.97
		Unsuppl	21.89	24.89	22.22	24.98	19.31	23.14
	St Dev	Suppl	7.96	8.12	8.18	7.89	6.11	8.19
		Unsuppl	7.64	8.27	8.40	8.09	6.34	8.19
	SE	Suppl	0.30	0.24	0.23	0.23	0.25	0.12
		Unsuppl	0.27	0.25	0.24	0.24	0.29	0.12
	P		0.02*	0.27	0.006*	0.02*	0.01*	0.33
Fat	Mean	Suppl	4.55	4.49	4.34	4.23	4.15	4.35
		Unsuppl	4.59	4.51	4.42	4.34	4.23	4.43
	St Dev	Suppl	0.81	0.74	0.73	0.76	0.61	0.75
		Unsuppl	0.74	0.78	0.73	0.84	0.59	0.77
	SE	Suppl	0.30	0.02	0.02	0.02	0.02	0.01
		Unsuppl	0.03	0.02	0.02	0.02	0.03	0.01
	P		0.29	0.44	0.01*	0.001*	0.03*	<0.001*
Lactose	Mean	Suppl	4.62	4.64	4.69	4.67	4.62	4.65
		Unsuppl	4.61	4.61	4.69	4.67	4.63	4.64
	St Dev	Suppl	0.28	0.25	0.28	0.26	0.26	0.27
		Unsuppl	0.27	0.27	0.26	0.23	0.27	0.26
	SE	Suppl	0.01	0.01	0.01	0.01	0.01	0.004
		Unsuppl	0.01	0.01	0.01	0.01	0.01	0.004
	P		0.54	0.01*	0.62	0.78	0.41	0.39
Protein	Mean	Suppl	3.41	3.47	3.45	3.50	3.34	3.45
		Unsuppl	3.45	3.48	3.44	3.50	3.29	3.45
	St Dev	Suppl	0.32	0.42	0.48	0.36	0.35	0.40
		Unsuppl	0.33	0.40	0.52	0.36	0.37	0.42
	SE	Suppl	0.01	0.01	0.01	0.01	0.01	0.006
		Unsuppl	0.01	0.01	0.01	0.01	0.02	0.006
	P		0.02*	0.67	0.67	0.55	0.03*	0.60
SCC	Mean[a]	Suppl	83.37	62.66	66.53	65.92	57.02	67.76
		Unsuppl	76.74	62.37	60.12	66.53	40.36	62.80
	St Dev	Suppl	4.15	3.13	3.65	3.25	3.40	3.40
		Unsuppl	3.33	3.01	3.54	3.26	2.92	3.23
	SE	Suppl	1.05	1.03	1.04	1.03	1.05	1.02
		Unsuppl	1.04	1.03	1.04	1.04	1.05	1.02
	P		0.22	0.93	0.05*	0.85	<0.001*	0.002*
Total	Yield	Suppl	16563.6	28440.8	26274.7	29624.1	11650.3	112553.5
	(Litres)	Unsuppl	16898.1	27538.5	26538.8	27425.8	9954.4	108355.6
		Total	33461.7	55979.3	52813.5	57049.9	21604.7	220909.1
	P		<0.001*	0.43	<0.001*	<0.001*	<0.001*	<0.001*

*P Significant, [a] Geometric mean. Suppl - biotin supplemented cows, Unsuppl - unsupplemented cows

CHAPTER FOUR

DISCUSSION

Trial design and procedure

The study was designed using epidemiological principles to carry out a prospective intervention field trial on the incidence of lameness and the effect of biotin supplementation on commercial farms. The allocation of animals to supplement group was successful. There were similar numbers of cows and heifers and similar numbers of days in the trial in each supplementation group.

The within farm design was very important because it reduced confounding between farms. The trial results confirmed its importance because there was great variation in the overall incidence of lameness and in the incidence of specific lesions between farms. This was an effect of different management systems, different housing systems, cow genetics and other unmeasured variables on each farm. These variations were controlled for in the within farm study design but have not been previously. Fitzgerald *et al* (2000) had a between farm design and therefore findings may have occurred because of farm effects or biotin supplementation, the two factors were totally confounded.

Herd Records

The ongoing herd records aided in the recognition that a good representation of animals were randomly allocated to each supplement. An interesting outcome was found when the number of calves and days to calving were analysed. More unsupplemented cows had only one calf compared to the biotin supplemented and

more supplemented cows had two calves than the unsupplemented. Farms 1, 4 and especially 3 also had a greater trial entry to calving time in the unsupplemented cows than the biotin supplemented cows. These findings may imply an effect of biotin on fertility.

Biotin supplementation

Some initial problems were encountered with biotin supplementation. There were electrical surges in the parlour system, which damaged the printed circuit board. This was repaired and prevented from recurring by the installation of an electrical protection system. A short time after the start of the trial the computer system was programmed to record the time and the number of doses given at each milking and whenever a fault occurred. This was considered necessary for accurate monitoring. Before the programme was ready this information was not known.

Physical damage to equipment also occurred when cows pulled pipe-work from the wall on occasions throughout the trial. Protective covers were fitted over the pipes to stop this on two farms and more 'heavy duty' fixings were required on one farm, all fittings successfully rectified the problem. Occasional problems occurred at other times in the trial, for example, a cow managed to damage a control panel in the parlour pit and stopped it from functioning and a water leak tripped the power to the storage tanks at one farm. In response to these problems the farmer contacted the researcher who rectified the problems or contacted the parlour system designer. Problems were usually rectified within a very short period (usually 1 day-1 week).

The farmers were initially concerned that supplementing cows using the control panel would be a laborious task which they would forget. However, after a short period they all found it to be a habitual process and thought they would miss it

when the trial ended. A regular turnover of students assisted on Farm 1 which was a problem because they required supervision at milking. This may explain why the supplementation was sporadic.

A double blind trial would have been preferable (Martin *et al* 1987), i.e. the farmers and veterinarians not aware of the supplement received. However the processes required to blind the farmers and veterinarians were considered too labour intensive and economically impractical because it would have been necessary to physically split the herd, which may have introduced additional confounders. It was considered to be more complex for a farmer to assert an influence and create a bias, in a busy day to day routine (Green *et al* 1997) than to simply supplement the cows with leg bands. The farmers were also aware that samples from both biotin supplemented and unsupplemented cows were taken to monitor biotin intake.

The use of leg bands for the identification of the supplemented cows was simple and successful. A few cows kicked when their leg bands were put on, but two bands were successfully attached to all of the biotin supplemented cows and heifers. There were a few losses, which were identified by the researcher (VJH) when monthly milk samples were collected. It was usually one band and since both legs were tagged replacements were applied immediately. The bands were easily seen even during times of heavy soiling, but became thin after a period of four or five months and needed replacing.

Biotin supplementation was given to the heifers 3 months before their predicted calving date whenever possible. Previous studies recommended the addition of biotin to the diet of heifers at least 60 days prior to parturition, because they only started supplementation at the start of lactation and believed that this had a significant

effect on their final results (Vermunt and Greenough 1990; Midla *et al* 1998; Fitzgerald 2000).

In the initial protocol the dry cows and heifers were to be supplemented with biotin via the water source at pasture or when housed. Experts advised that this would be unreliable because automatic refilling mechanisms would dilute the solution. It was also impractical to refill a tank manually as the daily consumption of water in the herd would, in many cases, exceed 200 times the capacity of the water tank. Biotin supplementation was given using dry cow rolls for the dry cows and heifers. This proved to be a successful alternative. The farmers initially reported that the cows showed a lack of interest in the rolls, which meant they would not eat the biotin. However, this was checked by the researcher (VJH) in the summer and winter months and the majority of the herd consumed the feed.

The feed was given to the cows by the farmer, or designated person on the farm, and even though this was more work, they accepted it as an important part of trial. It was imperative that the rolls contained the same ingredients, with the exception of the addition of biotin in the supplemented rolls, analysis confirmed a significant difference of biotin concentration. There were some initial problems with the preservation of the dry cow rolls. However, the problem was overcome early in the trial with the addition of a mould inhibitor. Any feed that appeared to contain mould was always discarded. All farmers were extremely cooperative and helpful.

For ease of feeding the dry cows and heifers were placed in different fields or housed separately around the farm according to their supplement, and on one farm, due to extreme weather conditions, animals were moved to a neighbouring farm. However, type of housing or field and their conditions and feed, apart from biotin in the supplemented animals, were almost identical. Any variability between biotin

supplemented and unsupplemented animals was controlled for more satisfactorily in the lactating herd where the cows ran together as one herd.

It was important that each farm was visited every week to monitor management and herd location changes and biotin consumption. It was also important that the tank containing biotin solution was refilled every week to keep the volume in the tank high, not only to ensure that enough biotin solution was available but also to ensure the heating element and pump that circulated the solution around the system were constantly immersed. On several occasions it was necessary to completely refill the tank after the cows had damaged and drained the system. These visits also proved to be an invaluable opportunity to maintain a very good relationship between the researcher and the farmers involved in the trial.

Farmer compliance was also successfully encouraged by the regular meetings between the research team, farmers and veterinarians. The farmers looked forward to these social occasions.

Biotin intake

Analysis of the milk samples confirmed the successful biotin supplementation of the study cows. Significant differences were observed between the cows receiving 20mg of biotin per day and unsupplemented cows and the concentrations found were similar to levels that have been previously described. These differences were observed at every level of analysis: cow, pooled samples and farm. They were consistent in three of the five farms.

The results from the analysis of biotin concentration in milk on Farm 3 were unexpected because, according to the computer records, the number of doses of supplementation was consistent. The biotin concentration in milk in the

unsupplemented cows increased quite considerably from the pretrial levels, by 96.1% in the analysis of pooled samples and by 141.43% in the analysis of individual samples. As the milk biotin levels were lower in the biotin supplemented group on Farm 3 than those observed on two other farms, but higher than the unsupplemented cows and the unsupplemented cows were higher than pretrial levels, it may be that some biotin was given to the unsupplemented cows. Although, it is likely that if this was happening that the herd would have much closer biotin values (Steinberg *et al* 1996). It is, therefore, more likely that cows receiving the supplementation did not eat all of their parlour feed and the unsupplemented cows ate a proportion. The fact that the milking herd received a mainly mineral based feed in the parlour during the summer months, that did not appear to be highly palatable, may explain this. This also confirms the success of having a secondary method for monitoring biotin supplementation.

Plasma biotin concentrations taken from dry cows and heifers were not as consistent as the milk biotin concentrations. The collection time of plasma samples were not standardised in relation to time of day and feeding. The veterinarians took the bloods during routine farm visits. The time of sampling in relation to feeding has a large influence on the amount of circulating biotin, however, utilisation by the animal has also been suggested to influence blood concentration and may vary greatly between individuals. Milk is more reliable for measuring biotin levels especially when intervals between sampling are long and only one sample per animal per day is taken (Klunter *et al* 1993; Steinberg *et al* 1996; Hochstetter *et al* 1996). Plasma biotin levels in the cow with and without biotin supplementation have also been analysed by several other workers who found highly variable results, some with no reliable

explanation (Frigg *et al* 1994; Klunter *et al* 1993; Hochstetter *et al* 1996; Voigt *et al* 2000).

When individual samples were analysed, biotin supplemented animals had significantly higher plasma biotin concentrations than the unsupplemented animals. This difference, however, was not significant in the pooled samples, but significant differences were observed between heifers and cows (heifers had higher plasma biotin values than cows) when supplementation and farm were also included in the analysis. It is proposed that this may reflect the size of the animals receiving the dose. Heifers are generally smaller than cows and may therefore comparatively receive a larger dose.

Interestingly, Farm 3 had the highest mean biotin plasma levels overall in both pooled and individual samples tested which may reflect the misallocation of biotin. As expected Farm 1 had a lower plasma biotin concentration than the other four farms because their supplementation was erratic and often insufficient.

The concentrations of plasma biotin on all farms in this trial were comparable with those reported by Frigg *et al* (1993) who observed heifers at the same supplementation rate, but were considerably higher than the concentration reported by Klunter *et al* (1993) who also supplemented cows at 20mg per cow per day.

<u>Lameness records</u>

Fifty eight percent of the cattle that took part in this trial were never lame during the 18 months. However, the lame cows contributed significantly more days to the trial than the cows that were never lame. No explanation can be found for this, although in a previous study lameness was more commonly observed in high yielding

cows than low yielding cows (Roberts and Baggott 1982) and may possibly reflect on the decision of the farmer not to cull high yielding cattle.

The farmers were very compliant in reporting lameness which was high at 68.9 cases per 100 cows per year, indicating that under reporting probably did not occur. Farms were not charged for the veterinarian visit and treatment. One farm decided to use their lameness data, which was not normally monitored so closely, to review their management system after the trial.

The lameness incidence of 68.9 per 100 cows per year, exceeds previous studies into lameness, the most recent being Clarkson *et al* (1996) who identified 55 new cases of lameness per 100 cows per year.

Locomotion assessment was used to monitor lameness in the herd by the trial vets and to identify any under-reporting or unidentified lameness. Where lame cows were identified in the locomotion assessment they had often been previously identified by the farmer and reported to the veterinarian, but had not been separated from the herd.

Lameness was recorded for an 18 month period per farm to capture more than whole horn replacement from the start of biotin supplementation. Therefore, cows that received biotin supplementation and remained in the trial for the full trial period had claws that had been fully replaced with horn potentially altered by biotin supplementation (Hochstetter *et al* 1996).

Impact of biotin supplementation on white line lesion lameness

The significant outcome of the trial was the reduction in the hazard of white line lesion lameness by approximately half with the supplementation of biotin. The

hazard for white line lesion lameness also increased with every lactation, although the confidence interval (CI) increased with the higher lactations as there were fewer cows and therefore less precision. Offer, McNulty and Logue (2000) also reported an increase in lesion numbers and poor locomotion scores with increasing lactation in their study and also significant effects of weeks post calving on lesion formation and claw conformation. In the current study, lameness was observed predominantly in the first 16 weeks after calving.

There were no interactions between farm and biotin supplementation and lactation and biotin supplementation. This highlights the consistent effect of supplementation across all five farms despite the obvious and wide variation in lameness, management and environment observed on each farm. This reflects a beneficial effect of biotin supplementation on these farms and an increased strength of the likelihood of the generalisability of biotin to reduce white line lesion lameness on farms with a high incidence.

The analysis was carried out initially with data from all five farms. However, because of the inconsistencies encountered on Farm 1, the data were also analysed excluding Farm 1 findings. The hazard for white line lameness was still reduced significantly as a result of biotin supplementation. Excluding Farm 1 reduced the cow numbers from 900 to 733 and the total cow years to 913.41 years, the statistical significance was not lost.

When Farm 3 was also excluded because of inconsistencies in biotin supplementation the trend was still for a reduced hazard of white line lesion but with a non-significant outcome because of the reduced power as the number of cows days were reduced.

The Kaplan-Meier curve of the crude effect of biotin supplementation on white line lesion lameness indicated that approximately 130 days of biotin supplementation was necessary before a divergence in white line lesion lameness was observed. Influences of biotin supplementation on hoof horn are not usually expected immediately, and the 130 days corresponds almost exactly with the time required for the white line (terminal horn) and sole horn to renew completely (Schmid and Geyer 1994; Zenker *et al* 1995). The horn leaflets of the white line originate from the coronary segment and take the same length of time to reach the bearing surface as coronary horn (Bolliger 1991). The adhesion between these layers with horn cells is also proposed to be slow (Budras *et al* 1989). These facts could account for the 130 days before divergence in hazard was seen.

Midla *et al* (1998) observed a significant reduction in white line separation (not lameness) in the hind claws of and lateral fore claw (left fore and right hind feet were tested) of first calving cows at day 108 following calving. A total of 100 cows were observed and supplementation started after calving, the difference was no longer significant by 293 days after calving. Fitzgerald *et al* (2000) also found a significant difference between biotin supplemented and unsupplemented cows in overall lameness score and clear differences were observed a year after the start of supplementation, although this was a between farm study on 20 farms and therefore prone to farm variations as mentioned previously.

Impact on the white line

White line lesions associated with lameness are more commonly situated in zone 3 of lateral hind claws (Logue *et al* 1998a), which is also the abaxial termination of the white line (Budras *et al* l996).

The horn produced in the white line is well known to be avascular (along with other horn material), high in turnover and incompletely keratinised and therefore, softer than horn in other sites. This makes it more susceptible to vascular disturbance, dyskeratosis develops and a reduction in claw quality and splitting of the structure results. Biotin plays a significant role in protein synthesis and the rate of sceloprotein (keratin) production and deposition (Roberts and Baggott 1982), as well as intracellular cementing substance (ICS). It is possible that additional biotin improves the function of enzyme systems, the structure and horn development or increase keratin proliferation and differentiation or both. Therefore improving the cohesion and adhesive function and quality of the white line horn (Fritsche 1990; Sarasin 1994; Hochstetter 1998), thus reducing the incidence of lameness as a result of white line lesion (Fritsche *et al* 1991; Midla *et al* 1998).

Fitzgerald *et al* (2000) found a reduced lesion severity as a result of biotin supplementation, however, the lesions in the current study were not scored for severity so an association cannot be confirmed. However, white line lesion lameness was re-examined more frequently in the unsupplemented cows than the biotin supplemented cows. A second visit to examine the same white line lesion may have reflected the severity of the lesion, so biotin supplemented cows would have had less severe white line lesions than the unsupplemented cows. Also in the secondary lesion data under

run sole, commonly associated as a further complication of sole ulcers, was more commonly observed in the unsupplemented than the supplemented cows.

Previous studies indicate that other lesions are influenced by biotin supplementation (Distl and Schmid 1994; Campbell *et al* 1996; Midla *et al* 1998). A reduction in sole ulcer lesions following biotin supplementation has been reported by Distl and Schmid (1994), although their study involved only 112 split by supplementation and Voigt *et al* (2000) observed a non-significant reduction but the occurrence of sole ulcers were too few (1.2% unsupplemented, 0% biotin supplemented). Sole ulcer was the most common lesion in the current study overall but biotin supplementation had no significant effect. This was similar to the study by Hochstetter *et al* (1996) which found unchanged incidence and severity of sole ulcer with biotin supplementation.

Recent findings in the pathological studies of sole ulcer by Ossent *et al* (2000) and Lischer *et al* (2000) suggests that an improvement of horn quality as a result of biotin supplementation may not be enough to overcome the pathologies that occur in the aetiology of sole ulcer and this may explain the varying results from different studies. Ossent *et al* (2000) and Lischer *et al* (2000) suggest that the supporting 'sling' structures in the foot, as previously described, are responsible for lowering the distal phalanx to cause pressure at the site of the flexure tuberosity. This also confirms recommendations that white line lesion and sole ulcers should be treated as separate lesions (Leach *et al* 1998; Logue *et al* 2000; Le Fevre *et al* 2001). In this study there was no evidence that these lesions were linked, because the incidence of the two lesions were very different on the farms and no pattern of claw distribution was observed to link them.

Farm 2, which had the highest incidence of lameness at 111.5 per 100 cows per year, already had a high incidence of lameness prior to the study. However, a track surface of sharp flint stones laid during the trial increased lameness caused by stone penetration and potentially white line lesion. There were fewer white line lesions in the supplemented animals (P=0.13) and significantly fewer foreign body penetrations (P<0.001). This may reflect the reduced cohesion or quality of the horn to withstand penetration. This incident also confirmed the importance of a within farm study.

An increase in white line lameness, and many of the other causes of lameness, were seen around the start of housing. This may be due to the procedures adopted by the farmer. Commonly the cows were not just removed from the pasture straight into housing, they were allowed access to the housing and pasture and this involved increased walking along different surfaces such as dirt and stone tracks and concrete yards. Previously, the cows would have only walked on a track onto concrete twice a day for milking. Once the cows were housed full-time the animals feet may have altered in response to the experience of the abrasive concrete for most of the day (Bergsten and Frank 1996).

An increase in lameness is commonly reported at housing. Studies from the Hannah Research Institute in Scotland, although small, have shown that housing, initiation of a silage based diet and calving in autumn, all of which were practised in the current trial, have led to a cumulative reduction in synthesis by laminar epithelium (Logue *et al* 1998a,c).

In this period the cows' routine changes considerably. Initially, behavioural dominance characteristics become more prominent and for example, prevent cows from lying down. These have been found to have a profound effect on lameness in other studies (Baggott and Russell 1981; Colam-Ainsworth *et al* 1989; Singh *et al*

1993). Nutritional changes from natural grazing to increased concentrate rations (Livesey and Flemming 1984; Manson and Leaver 1988a,b; Manson and Leaver 1989; Chamberlain and Wilkinson 1996; Livesey *et al* 1998a; Livesey *et al* 2000b), housing type (Vermunt and Greenough 1996a,b; Smilie *et al* 1999), floor characteristics change for example from grass and earth to concrete, straw and slurry (Baggott and Russell 1981; Russell *et al* 1982; Distl and Mair 1990) also affect lesion incidence. These factors were evenly represented in biotin supplemented and unsupplemented cows in the within-herd design. All of the above factors, and the association between walking and white line penetration, have been identified by many workers as large risk factors for lameness which can be complicated further by calving season which varies in practice and time of year for each farm (Livesey and Fleming 1984; Peterse 1985; Manson and Leaver 1989; Andersen and Jarlov 1990; Distl and Mair 1990; Tranter and Morris 1992; Blowey 1993; Mortensen 1994; Ossent and Lischer 1994; Vermunt and Greenough 1995a,b and 1996a,b; Greenough and Weaver 1997).

Milk quality

Somatic cell counts (SCC), were overall quite low, on Farm 3, Farm 5 and all farms combined they were significantly higher in the biotin supplemented than the unsupplemented animals. This is unlike the findings of Fitzgerald *et al* (2000) who found a significant reduction in SCC as a result of biotin supplementation.

Midla *et al* (1998) found improved milk yield in first lactation cows supplemented with biotin, but Fitzgerald *et al* (2000) did not find any improvement in milk yield. Weiss and Zimmerly (2001) reported a significant linear increase in milk protein and milk yield in the first 100 days of lactation in biotin supplemented animals

compared with unsupplemented. In the current study, despite the differences found on individual farms, no obvious differences in the milk protein and total and mean monthly milk yield were found in biotin supplemented animals.

However, the milk fat content was overall quite high and significantly higher in the unsupplemented cows of 3 farms and in pooled farm data. This is similar to the findings of Fitzgerald *et al* (2000) who also found a reduction in milk fat as a result of biotin supplementation.

CONCLUSION

The current trial provided evidence that in a commercial situation where cattle are exposed to many factors that may influence the occurrence of lameness, it is possible to run a controlled intervention study. The incidence of lameness, 68.9 per 100 cows per year far exceeded previous estimates. Biotin supplementation at 20mg per cow per day, significantly reduced the hazard of lameness caused by white line lesion, by approximately half. The precise mode of action of biotin on the white line region is still unknown, however, this trial highlighted that farms with a high lameness incidence as a result of white line lesion could benefit from supplementing cows with 20mg biotin per cow per day, alongside a review in management, housing and environment.

CHAPTER FIVE

Tensile strength testing of the white line and microhardness testing of the sole adjacent to the white line on the claws of dairy cows supplemented with and without biotin

BACKGROUND

White line structure and function

The region of the cow's foot containing the white line (zona alba) lies between or joins the harder structures of the wall horn and the relatively pliable sole horn. It runs from the heel bulb around the distal abaxial portion of the claw to the toe and along the anterior third of the axial wall. It then continues in a vertical direction up the inside of the claw (Martig *et al* 1980; Vermunt 1990; Blowey 1993; Blowey and Greenough 1998). The white line is quite easy to distinguish from the coronary horn with the naked eye, but white line tubules run into the sole horn tubules and this makes it difficult to identify the precise demarcation of the white line to the sole, although the sole material is often more cohesive (Warzecha 1993).

The white line is commonly referred to as the 'hinge' region (Budras *et al* 1996, 1998b). It is relatively weak compared with the other structures. The most vulnerable sites are the axial and abaxial terminations (Figure 5.1). It is also quite susceptible at the join with the coronary horn because of the extreme differences in their structure (Budras *et al* 1996). The white line develops 1-2cm proximal to the outer or ground contact surface of the hoof (Warzecha 1993), with formation

exclusively from the wall segment (Budras *et al* 1996). Consequently the tubules are short and damage and infection are comparatively easy (Budras *et al* 1998b).

Budras *et al* (1996) identified three sections to the white line in a horizontal plane (3mm width) (Figure 5.2) (Budras *et al* 1998b). The outer part consists of cap horn and the basal portion of the horny laminae alternately interlocking, which are bound to the inner tubule free layers of the stratum medium unguis; the mid portion alternates with distal cap-horn arches and the mid portion of the horny laminae; the inner structure has apical portions of horny laminae alternating with large terminal tubules and intertubular horn. Only one horn tubule type exists in the cow (Warzecha 1993). The apical portion of the horny laminae appear distinctly in the white line at the abaxial termination. In this region the horny laminae travel higher into the foot and their length is shorter than in any other part of the white line; it is the region of highest horn production (Budras *et al* 1996).

The dermal laminae are living tissue so they do not reach the volar border, terminal horn tubules and cap horn continue the structure distally to the border (Budras *et al* 1996, 1998b) (Figure 5.2).

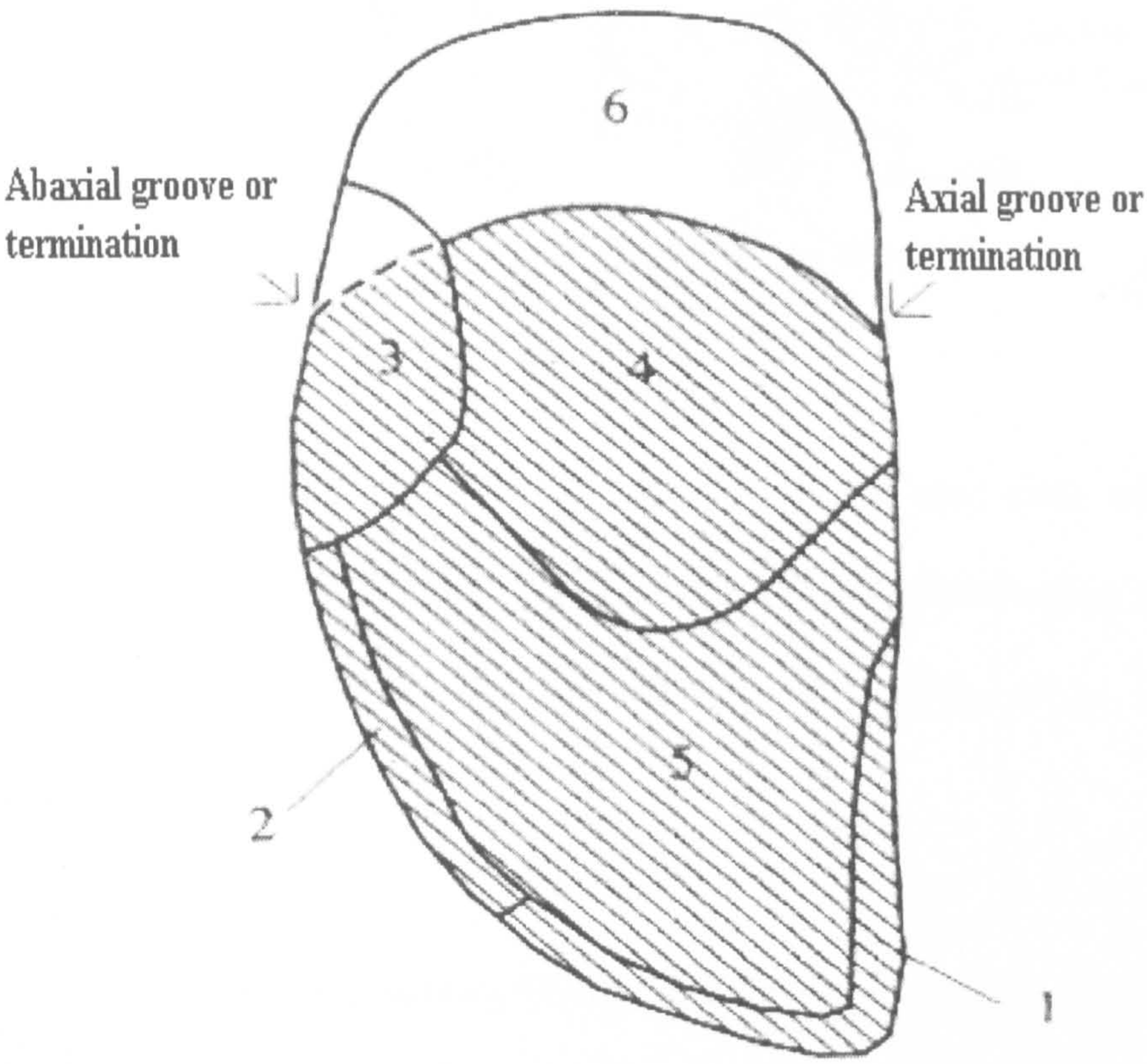


Figure 5.1: Zones of the distal surface of the claw, volar aspect. 1, white line at the toe; 2, abaxial white line; 3, Abaxial wall/bulb junction; 4, Sole/bulb junction; 5, Apex of sole; 6, Bulb of heel, Abaxial groove-abaxial termination of the white line (taken from Leach *et al* 1998).

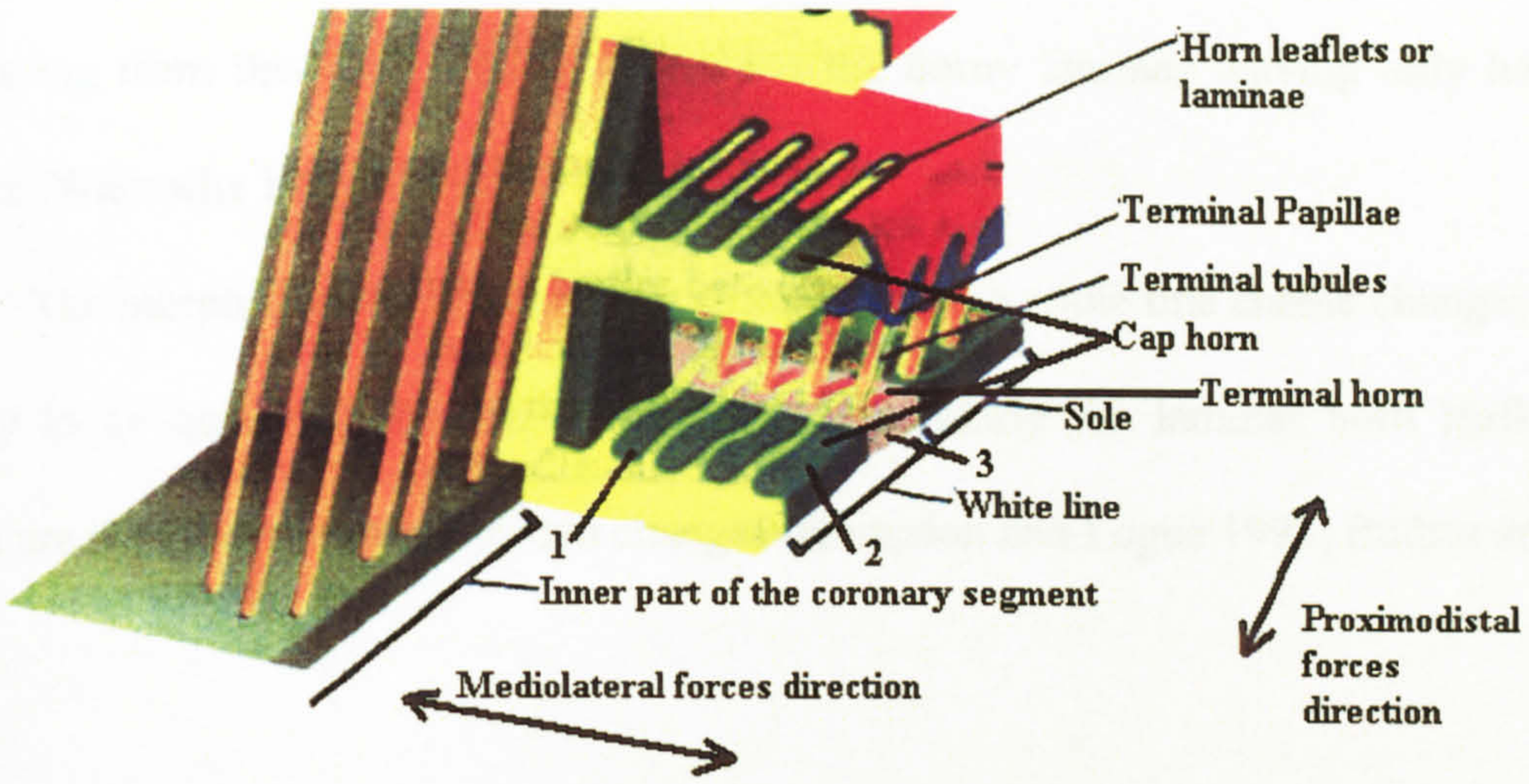


Figure 5.2: Structure of the distal portion of the bovine claw with the principal directions of locomotion forces with the addition overall of radial tension. 1, Outer white line; 2, middle white line; 3, inner portion of the white line (taken from Warzecha 1993).

The white line consists of a small layer of horn leaflets and approximately 75-80% cap and terminal horn which serve as the 'sealant' to the claw and resistance to ascending infection (Budras *et al* 1996). The cap horn is produced over a narrow stratum basale and originates without any stratum granulosum and is therefore soft and bilaterally pliable and compressible, which allows changes in claw shape during weight bearing and locomotion (Kempson and Logue 1993). The cap horn of the cow is very well developed (Kempson and Logue 1993) but terminal horn is produced over a small surface of the corium and cannot withstand much force (Warzecha, 1993) but has an elastic role between the stabilising laminae (Budras *et al* 1998b).

The white line shows the most intensive production of incompletely keratinised hoof horn with broad spaces containing intercellular cementing substance, therefore the structure is less stable and more susceptible to damage (Budras *et al* 1996, 1997) than the sole or coronary horn. Often the cap horn and terminal horn can

be missing from this site or fall out between the horny laminae leaving only horn leaflets (Warzecha 1993; Budras *et al* 1996).

The morphological differences in structures in the white line enable changes in quality to be quickly and reliably identified, particularly the laminar horn leaflets which are the first to show abnormal changes (Kempson and Logue 1993; Budras *et al* 1996).

Keratin in biomechanics

The main constituent of the bovine hoof is keratin. The advantages of keratin, as a mechanical design material have not been widely investigated (Vincent 1992) but in hoof horn of the dairy cow and other species its properties are well suited for the multidirectional and unpredictable forces applied during locomotion (Bertram and Gosline 1986; Bonser 1999). The hoof can deform, both highly elastically and plastically and therefore requires greater material energy and strength e.g. 2000 times stronger than glass, as glass cannot deform elastically. It is one of the toughest biomaterials known (Bertram and Gosline 1986). A small amount of keratin biomechanical research work has studied the horse hoof but surprisingly, very little work has been carried out on the dairy cow foot.

The important functions are hardness, toughness and viscoelasticity, which depend on structure, chemical composition of the keratin forming the horn and amorphous material present (Baggott *et al* 1988; Vermunt and Greenough 1995b).

Keratin is a three dimensional composite material. It consists of slender ∝-helical protein fibres and an amorphous protein matrix that results in mature flattened discs with extensive molecular crosslinking. This produces a stable composite. The

production of keratins start with the replacement of cell contents with keratin proteins, these proteins are then organised into tonofilaments by the combination of two molecules (one of each subtype) to form a coil. This combines with another coil to form a four poly-peptide complex, and subsequently they are incorporated into the cell cytoskeleton and stabilised with di-sulphide crosslinks and hydrophobic interactions (Bertram and Gosline 1986; Budras *et al* 1989; Kempson and Logue 1993). The tonofilaments are aligned in the cells and linked to their neighbour (Bertram and Gosline 1986; Budras *et al* 1989; Leach 1993). Lipid-rich membrane coating material (Baillie *et al* 2000) is then secreted in which mature keratinocytes become embedded (Elias 1981). Once the fibres and matrix are established the cells that synthesise keratin die (Bertram and Gosline 1986).

The high rate of horn production, at a site where the marginal artery is smallest, also makes the keratinising portion of the epidermis susceptible to vascular disturbances which lead to structural alterations and a depletion in horn quality (Budras *et al* 1996). The keratinising medullary cells of the tubules are more prone to receive less nourishment and few keratin filaments. Reduced nourishment also potentially occurs in the intercellular cementing substance which cannot 'adhere' as effectively. Therefore, due to the lack of stability in this structure any slight insult can potentially result in the disintegration of the horn cells in the centre of the tubules (Budras *et al* 1998b). The outer (cortical) cells of the tubules, however, have enough time, in cell replacement, to keratinise the entire surface of the dermal papillae. The tubular cortex is therefore a stable structure and the intertubular horn has moderate strength. The overlap of keratin cells around the tubules and the spaces in the protein matrix is also thought to play a key role in regulating horn hydration (Baggott *et al* 1988; Baillie *et al* 2000).

Epithelia contain keratins that differ in expression. This also includes diseased horn which is distinguished by unusual keratin expression (Hendry *et al* 1997). Although, Meyer *et al* (1997) suggested that bovine sole horn keratin expression does not differ between lame and healthy horn. However, Budras *et al* (1996) recognised that in the case of laminitis or coriosis, the white line expands in response to increased keratinisation, which increases the proportion of elastic, weak, terminal tubular horn between the horny laminae, which are pathologically altered, and an inferior non-adhesive intercellular cementing substance. This combination cannot sufficiently deter decay (Budras *et al* 1998b). Johnston (1990) showed that the normal organisation of keratin fibres was disrupted and irregular in electron microscopic examination of white line slivers from pre-calving heifers with poor quality horn.

Biomechanics of the bovine claw

A healthy claw or foot provides a barrier for the underlying tissues and protection against external trauma and environmental factors. Baillie *et al* (2000) identified that the normal healthy bovine claw had very good mechanisms for protecting itself against fracture at different levels of moisture. Stability was found as a result of the previously described horn structure, its orientation of tubules and in relation to the intermediate filaments in the intertubular material. Hoof material is not uniform throughout, there are differences in the water content, histology and cytology organisation and pigment. As a result, different parts of the hoof perform differently (Leach and Zoerb 1983). It may be presumed therefore that the structure of the white line is different biomechanically to the rest of the claw to fulfil its function (Leach and Zoerb 1983).

Normal locomotion transfers the body weight of the cow from the distal phalanx into tensile forces, which act on the interdigitating or epidermal and dermal attachments to the outer hoof wall, which is converted to pressure on the ultimate weight bearing site in the sole border and heel (Warzecha 1993). The white line, as previously stated, is the 'hinge' between these structures (Budras *et al* 1996, 1998b).

A complete healthy horn structure is important in providing support, which effectively aids in the dissipation of stress and the weight of the body over the hoof filaments during locomotion (propulsion and concussion), modulate any irregularities in the applied load (Johnston 1990; Vermunt and Greenough 1990) and provide resistance to excessive abrasion (Leach and Zoerb 1983; Douglas *et al* 1996). Factors that may effect the required qualities include, size of the claw, posture and gait (Distl and Mair 1990), the density of tubules, the digital cushion and abaxial wall and sole structure, all of which may assist in abnormal function or loading and potential damage (Kempson and Logue 1993; Phillips *et al* 1996).

Shearing forces, due to hoof quarter expansion during locomotion in the horse have been reported to form clefts in the laminae and terminal tubular horn. Expansion also occurs in the bovine claw (Baggott and Russell 1981; Greenough *et al* 1997). Inadequate exercise or uneven or abnormal limb loading may also result in the propagation of cracks (Johnston 1990; Vermunt and Greenough 1990).

The claws of dairy cows are subjected to mechanical and environmental stress. Stress may be as a result of factors that have been previously outlined in the main literature review. These factors have a large influence on the growth:wear ratio, locomotion forces, hardness, water content and therefore the chemical and mechanical properties of the claw (Vermunt and Greenough 1995b; Sugg *et al* 1996).

It is important to understand the biomechanical properties and the anatomy of

the foot to identify the function of different parts.

Mechanical testing and claw hardness in horses

In studies carried out on horses, previous history of the horn has been identified as a significant factor effecting the results of stress and strain behaviour in radial tension and proximodistal and mediolateral shear of the laminar junction (Figure 5.2). Although the shape of the foot does not effect the results (Douglas *et al* 1998). In support of other theories into this biomaterial, Douglas *et al* (1996) found that the structure of normal healthy horse hoof, with its multidirectional and elastic properties, are specifically and ideally designed to allow universal movement during normal weight bearing and locomotion in a highly changeable strain pattern.

Like locomotion in the cow, the wall of the horses hoof is loaded (compressed) in the stance phase of locomotion. However, each limb of the horse has a hoof and not claws. So the hoof deforms at the quarters (side wall) so that the proximodorsal wall rotates caudo-ventrally about the distal dorsal border with lateral-medial flaring at the back (Douglas *et al* 1996). Wear of the hoof occurs from the oldest and most fatigued material and the horn organisation means that it wears in the least detrimental direction. This process helps to reduce the propagation of micro-cracks (Bertram and Gosline 1986; Thomason *et al* 1992).

Most of the work carried out on horses and cows has focused on the coronary horn. Douglas *et al* (1996) studied, in the horse, both compressive and tensile strength around the hoof both proximo-distally and circumferentially. Compression tests found significantly stiffer outcomes (Megapascals) than tensile strength, which may have been the product of a faster strain rate in compression than tension because of the

viscoelastic properties of the hoof.

Douglas *et al* (1996) and Leach and Zoerb (1983) found differences between the outer dorsal wall, which had a stiff structure (moisture approximately 23%) and the inner dorsal wall (moisture content approximately 30%). Moisture content alters marginally as a result of seasonal humidity changes and environment, for example, wetness of floor surface (Buffa *et al* 1992). Cows do not have such a distinctive difference, which is discussed later.

The difference in the inner and outer structures is believed to be for inner claw protection (Douglas *et al* 1996). The higher level of hydration is thought to be required to provide a suitable interface for the dermis and epidermis to minimise the stress of concussion and as it is located next to tissue which is fully hydrated, it may extract some hydration from the inner structure (Douglas *et al* 1996). Leach and Zoerb (1983) also believed that moisture altered the stability of the ∝-helical microfibrils of the keratin fibre.

With tensile strength testing it is possible to identify the presence of micro-cracks in the coronary horn (Douglas *et al* 1996). These cracks have been associated with the most distal wall material (1 cm from the bearing surface) than with more proximally located horn. A significantly weaker fracture toughness was identified by Bertram and Gosline (1986) in the distal wall area, half the value of the proximal horn. These micro-cracks were associated with fatigue as a result of age and commonly associated with higher amounts of moisture. Therefore damage was more evident in the stratum medium and inner structures (Bertram and Gosline 1987; Kempson 1987; Josseck *et al* 1995; Zenker *et al* 1995). Landeau *et al* (1983) reported a higher compressive modulus in distal wall samples compared to proximal samples. Another study by Douglas *et al* (1996) did not find the same results, however, their

distal samples were removed more proximally than that of Landeau *et al* (1983).

A significant difference in pigmented and non-pigmented horn has not been found, in the horse, for penetration hardness, water content and chemical composition (Leach and Zoerb 1983), or compressive stiffness and ultimate compressive strength (Landeau *et al* 1983), or fracture toughness (Bertram and Gosline 1986; Douglas *et al* 1996).

Work has been carried out on horn at different orientations to identify the potential elasticity, rigidity, fracture propagation and strength in different directions. Leach and Zoerb (1983) tested horn elasticity or rigidity and yield point in a lateral and vertical orientation by compression. Lateral loading had significantly greater values in elasticity and yield than vertical loading in the outer stratum medium tissues. No significant difference was observed between vertical and horizontal compression in the inner tissue.

In samples taken from horse hoof horn, alongside and perpendicular to the tubules, Douglas *et al* (1996) found that the structure of the tubules and intertubular horn components ran at right angles to one another, except in the quarters (side wall) where no anisotropic behaviour was found, defined as different physical properties in different directions (Bertram and Gosline 1986). When testing was carried out in the dorsal horn on a tubular and intertubular axis, anisotropic behaviour occurred in tension only and dorsal wall stiffness was greater in parallel than perpendicular loading. This was unlike the findings of Bertram and Gosline (1986) who found greater stiffness and strength in perpendicular loading by 18% and 9% alongside the tubules.

Bertram and Gosline (1986) also found that there was a statistically significant relationship between fracture propagation and the orientation of the intertubular cells,

fracture growth was more apparent parallel to the intertubular cells. In samples notched perpendicular to the intertubular horn the fracture course changed direction to run parallel to the intertubular horn. Therefore, fracture behaviour was dominated by the intertubular material and its orientation. The strongest fracture resistance was parallel to the tubules (Bertram and Gosline 1986). This intimates a method of crack prevention proximally built into the horn structure. Any crack or damage would run on an intertubular plane parallel to the ground surface and halt at tubules, which prevented the crack from continuing around the entire hoof. Therefore both structures play a very important role. Tensile strength of the wall horn of horses was largely similar in the two different orientations (parallel and perpendicular to the horn tubule) (Bertram and Gosline 1986).

Biotin supplementation and biomechanics in other species

Biotin supplementation has been studied as a method for improving the mechanical strength of horn, which has been broadly discussed previously. The following section contains a summary of specific biomechanical studies on biotin supplementation in species other than the cow.

Zenker *et al* (1995) noted a loss in cohesion of the cells in the white line material of horses especially from terminal to leaflet horn and Josseck *et al* (1995) postulated that the mode of action of biotin may be in improvement of the intercellular cementing substance or the avoidance of the premature decay of horn cells. Bendit and Gillespie (1978) reported an improved transverse compression elasticity, which they correlated with the number of high sulphur and high glycinetyrosine proteins present in the matrix between keratin fibres. Webb *et al* (1984) believed that biotin

supplementation affected the number of these proteins present.

Josseck *et al* (1995) carried out a whole hoof evaluation and identified an improvement in horn quality macroscopically after 9 months biotin supplementation in Lippizaner horses. Zenker (1991), however only saw a slight improvement in tensile strength following biotin supplementation (20mg/head/day) after 19 months, further improvements were noted only after 2 years of supplementation as the placebo group values depleted. However, a later study by Zenker *et al* (1995) observed higher tensile strength than the placebo at 33 and 38 months with biotin supplemented horses.

Significant improvements were seen in hoof horn hardness by Buffa *et al* (1992) after 2 months of 15 mg per day biotin supplementation (sharp tipped probe). Growth rate of the equine hoof, specifically in the quarters (P<0.01-0.04) and toe region (P 0.02) of the hoof has been reported to increase in response to biotin supplementation. This result was similar to the findings in the pig after biotin supplementation by Webb *et al* (1984) who found with biotin supplementation, 1mg d-biotin/kg feed, significantly increased hardness in the mid abaxial region ($P<0.05$) and improved compressive strength by 2.7×10^6 N/m^2 (9.3×10^6 N/m^2 improving to 12×10^6 N/m^2). The dorsal border was significantly harder than the abaxial mid region ($P < 0.001$) and had a higher compressive strength of $14.5 (\pm 0.7) \times 10^6$ N/m^2. Quality of these regions were not improved by biotin supplementation. Buffa *et al* (1992) and Webb *et al* (1984) only tested the horn material in parallel to the tubules, i.e. in one direction.

Mechanical testing and claw hardness in the cow

Although many observational studies have observed the growth and wear rate of the dairy cow claw (Tranter and Morris 1992; Vermunt and Greenough 1995b; Offer, Logue and Leach 2000; Livesey *et al* 2000a), fewer studies have successfully detailed dairy cow claw micro-hardness and mechanical properties. This is surprising when considering the significant effect of lameness on loss of production, welfare and the large number of lesions observed (Clarkson *et al* 1996; Leach and Zoerb 1983). Many similarities can be drawn between the horn biomechanics found in horses and cows, which may be utilised in further research work.

Mechanical tests

The normal tensile strength of coronary horn in cows (70.6 N/mm^2 in normal abaxial horn, 72.5 N/mm^2 for axial horn) has reported to be higher than those observed in the horse or pig (64.7 N/mm^2 abaxial horn). Normal sole horn tensile strength is softer in cattle than coronary horn but comparable to the coronary horn of pigs and horses (64.7 N/mm^2). Bovine heel horn is even softer because of its biomechanical role (59.8 N/mm^2) (Bohli, 1993; Albarano 1993). In the white line of cattle Budras *et al* (1996) used ball impact methods, which identified average values of 5.1 N/mm^2 for the middle part (cap-horn the main portion) and 6.9 N/mm^2 for the inner part (terminal horn the main portion). Sole horn in comparison was 12.9 N/mm^2 and coronary horn 27.5 N/mm^2.

Similar behaviour in bending response was seen in bovine horn to that in other species 410MPa (100% hydration) to 14,600MPa (0% hydration). As expected, a more brittle behaviour is found in the drier material and a more ductile or soft

behaviour in the wetter material. Moisture content plays a much more significant part in this test procedure than the direction of the horn material. No significant change in the behaviour of the horn material was found in relation to moisture content until this dropped well below the level (25%) that would be expected in horn *in vivo* (Baille *et al* 2000).

In cow claw horn double ended notch test and wedge tests, crack propagation has been found to take a more brittle straight path when tested alongside the tubules and a very diverted path when perpendicular to the tubules. This indicates that the intermediate filaments are largely involved and the orientation of the tubules play a major part in crack propagation, failure paths try to avoid breakage of fibre or tubular structure (Baille *et al* 2000). The same behaviour that has been observed in the horse hoof (Bertram and Gosline 1986; Baillie *et al* 2000).

Hardness

More work has been carried out investigating hardness of bovine hoof horn than any other biomechanical tests and most testing has been carried out using shore durometers.

Hardness is generally assessed as the resistance of a material to penetration by a harder object. This measurement is very dependent on water content and how this relates to micro-architecture, biochemical composition of the horn and overall quality (Vermunt and Greenough 1995b).

Hardness is related to toughness or resistance to wear, the harder the material the greater its resistance to wear (implies resistance to flow and plastic deformation). Wear can also be regarded as a process of microfracture. Hardness is not related to stiffness although stiff materials are concerned with wear resistance (Vincent 1992).

Hardness is greater in the coronary horn (mean 76 shore D degrees) than the sole horn (mean 48 shore D degrees) but dependent on humidity (Schmid and Geyer 1994). There is disagreement over whether fore and hind limb hardness differs (Martig *et al* 1980; Distl *et al* 1984). Soft wall horn may, however, be more resistant to the abrasive wear of concrete because of its ability to expand and contract, whereas a soft sole provides little protection to the underlying sensitive structures in locomotion (Vermunt 1990).

Unlike the horse, cow coronary horn has the same moisture content, therefore hardness, throughout (Vermunt and Greenough 1995b), which fluctuates (Distl *et al* 1984) and increases with age. Baggott *et al* (1988) found an increase of 5% moisture from 2-4 years of age. Other factors that vary with water content and are inversely correlated to hardness are potassium, iron and sulphur horn concentration (Baggott *et al* 1988).

Martig *et al* (1980) did not find a difference in the hardness or moisture content of horn between different claw lesions. However, Maclean (1971) and Vermunt and Greenough (1995b) reported high moisture content in laminitic horn which was deemed responsible for the laminitic outcome. A higher water content and softness was observed in the hind claws opposed to the front (Martig *et al* 1980) which may explain the greater incidence of lameness observed in hind claws of cows (Hedges *et al* 2001). This could however, be a result rather than a cause.

Overall it is the opinion of most that high pigmentation indicates good quality horn and less pigmentation indicates poor quality horn (Vermunt and Greenough 1995b). Studies in melanic (pigmented) starling beaks found an increased hardness by 39-69% (Bonser and Witter 1993). Pigmented horn in cows has been reported to be 30% harder (Feder 1969 cited by Vermunt and Greenough 1995b), although there are

conflicting studies. Clark and Rakes (1982) and Douglas *et al* (1996) found that hardness was unrelated to colour. Others have identified an increased in severity of certain sole lesions with less pigmentation. Chesterton *et al* (1989) found that in high-lameness prevalence dairy herds it was more likely that, on average, the claws of the cows were less pigmented and that the percentage of Jersey-type cattle was low.

There are several factors that could potentially reduce the hardness of the cows claw in addition to the direct effect of moisture levels. Hardness may be reduced by a lack of nutrients (Vermunt 1990), a high concentrate: silage diet (Manson and Leaver 1989) and urine and faeces, which also reduces tensile strength (Albarano 1993). Clark and Rakes (1982) found reduced horn hardness when methionine was added to the diet and they postulated that this may be a result of reduced disulphide bonding and less cysteine, in the supplemented cows. Johnston (1990) found an increase in horn hardness in cows supplemented with biotin.

Claw trimming has been reported to reduce horn hardness of the heel bulb, but this may be largely due to the shift of weight bearing over the foot reducing the use of the heel (Manson and Leaver 1988b; Phillips *et al* 2000). As a result of reduced heel use Manson and Leaver (1988b, 1989) also found an increased mid-sole hardness, although it was not significant, and the mid-sole area is softer generally than the toe-sole region. However, a significant negative correlation was observed between mid-sole hardness and locomotion score indicating the importance of sole horn hardness in lameness occurrence.

INTRODUCTION

Biotin intervention studies in dairy cow biomechanics

In a recent study (Hedges *et al* 2001) we identified that dairy cows supplemented with 20mg biotin per day had a reduced risk of lameness caused by white line separation of 0.59 fold. One hypothesis for this reduction in lameness is that the biotin supplement strengthened the cellular and intercellular tissue adhesion of the white line making a healthy defined and more cohesive structure (Mulling *et al* 1999, Hochstetter 1998; Fritsche, 1990; Johnston, 1990), through its function as an essential nutrient in keratin synthesis and lipogenesis (Sarasin, 1994; Whitehead, 1988) and influences on proliferation and differentiation of the epidermis (Fritsche *et al* 1991; Sarasin, 1994).

Very few studies exist that examine the effects of biotin supplementation on the biomechanics of the dairy cow claw.

Several studies have observed hardness in relation to biotin supplementation. An increase in horn hardness, particularly in the lateral wall, was found in 56 cows by Distl and Schmid (1994). Schmid and Geyer (1994) also identified a slight but not significant increase in hardness. Humidity or moisture content in the coronary and sole horn also showed a negative correlation. Hochstetter *et al* (1996), however, did not find an effect. Many measurements were taken by a shore C-durometer and any differences were attributed to the housing surface.

Schmid and Geyer (1994) also studied the tensile strength in bovine heel and sole material of 5 dairy cows monitoring growth rate, shore D hardness and moisture content. Five months after the beginning of the trial significant differences were

observed in quality and tensile strength of the sole (apical heel) and heel (apical heel increased from 53 to 65 N/mm^2, heel bulb region increased 40 to 55 N/mm^2). This was observed after 10 months in the coronary horn (means of 67.7 N/mm^2 which increased to 70.7-74.2 N/mm^2 after biotin supplementation). Moisture content and any growth was not associated with dietary biotin. In a study by Reilly and Brooks (1990) biotin supplemented animals experienced greater net horn growth. Their study was however over 4 months with a total of 36 cows split to supplement.

Due to the significant reduction of white line lesion lameness in the recent trial as a result of biotin supplementation (Hedges *et al* 2001), further work was carried out to test the hypothesis that the white line structure was strengthened from biotin supplementation. Tests were carried out on horn samples using biomechanical techniques for testing white line tensile strength and micro-hardness of the sole adjacent to the white line.

MATERIALS AND METHODS

Farm and sample allocation

The intervention study was carried out from April until November 2000 on a commercial, family managed farm in the Forest of Dean, Gloucestershire. The farm had approximately 60 milking cows. During the study the cows were at pasture and walked a small distance no greater than 400 metres on a stone track, or combination of track and tarmac, into a concrete yard to be milked twice a day and received a small amount of concentrate within the parlour.

Heifers and first lactation cows with no previous history of clinical lameness were selected for this study. The number of cows in each group was estimated to include the minimum number of cattle to detect a significant difference using the Wilcoxon rank sign test (Kirkwood, 1988). Six cattle were required in each group. A total of 14 dairy cows were selected to allow for cow losses during the study. All cows were stratified by calving date and then allocated randomly to the two groups: biotin supplementation or no supplementation (Martin *et al* 1987).

The farm had an abreast milking system which made supplementation with the biotin supplemented or unsupplemented feed possible. All cows received concentrate in the parlour at milking and the cows consumed their ration readily.

For ease of identification the 7 cattle that received biotin were identified with yellow tape wrapped around their tail in two bands. The seven unsupplemented cattle were labelled with red bands. This corresponded with feed bag and bin identification.

Biotin supplementation

The biotin supplement was included in the concentrate feed at a rate of 20 mg per kg. Two batches of feed were manufactured by Roslin Nutrition, UK and dispatched to the study farm (feed ingredients are located in Appendix II). The feeds were identical in composition with the exception of additional biotin to one ration. The feed was delivered in paper bags and stored under cover to protect from environmental damage. The quantity of feed consumed was monitored.

To make supplementation easier and less labour intensive, the feeds were stored in separate plastic dustbins in the parlour to protect the contents from damp and vermin. The feed bags in the barn and bins in the parlour that contained biotin supplemented feed were labelled with yellow tape and bags and bins that contained non-supplemented feed were labelled with red tape.

Both bins contained a measuring tin calibrated to 0.5kg by the researcher (VJH), which were also marked with coloured tape respectively. Each cow was given 0.5kg of feed twice a day at milking. So each biotin supplemented cow received a total of 20mg of biotin a day.

Biotin concentration monitoring

Biotin concentration was monitored in milk samples collected from 8 randomly selected (4 per group) study cows before supplementation started and two and fourteen weeks after the start of supplementation. Not all cows were sampled. The composite sample of approximately 50ml was taken from the collecting jar following the removal of the cluster and therefore comprised a mixture of milk from all four quarters of the cow. These were stored at -20^{0}C before being packed on dry ice and

transported to F Hoffmann-La Roche Ltd., Basle, Switzerland for biotin microbiological assay analysis using *Lactobacillus plantarum* ATCC 8014 (F. Hoffmann-La Roche Ltd).

Lameness reporting procedure

When the farmer observed a lame cow he reported it immediately to the researcher and the veterinary surgeon who attended the animal without charge to the farmer. One veterinarian attended all of the lame cows. Each lameness was documented with details on the date, cow identification, lesion identification and location and any treatment applied (same form as Hedges *et al* 2001, Appendix I).

Hoof sample collection

Samples of horn were collected on day 0 (sample 0, April/May), 53 (sample 1, July), 90 (sample 2, August), 117 (sample 3, September), 147 (sample 4, October) and 187 (sample 5, November). Four cows were culled during the study leaving 5 cows per group from day 117 onward.

The very outer, environmentally contaminated or damaged slivers of horn were removed by skimming the surface with a hoof knife. Horn samples were taken from zones 2 and 3 (Figure 5.1) of the volar surface from both lateral and medial claws of the left and right hind foot. A total of eight samples were collected from each cow at each visit. Samples were removed by the same veterinarian at each visit, using a hoof knife along the volar aspect under the surface of the claw to a depth of a maximum of 2 mm. The samples were placed in separate sterile pots containing de-mineralised water, labelled and stored frozen at -20^0C.

Tensile strength testing

The strength of the white line was measured by treating it as an adhesive layer. To ensure that we measured the failure properties of the white line rather than adjacent wall or sole tissue, we elected to test 'notched' specimens (Vincent 1992), which involved a slither of a rectangle of material in a horizontal plane, so that the wall material was at one end and the sole at the other and the white line ran the width of the rectangle, the notches were taken out of either side of the white line material.

Frozen specimens were thawed at room temperature and maintained in a wet condition during preparation. The sample was inspected for the presence of necrotic debris or blood invaded white line material, which was recorded as white line damage. The thawed hoof horn was cut with a specially designed cutting instrument A to a standard width and 'waisted' by a second cutter B (Appendix V) in the centre of the white line region in a cranial/caudal plane. These cutters were used to control the relative notch width to remove the possible confounding influence of notch-sensitivity in the material (Vincent, 1992). The thickness of each specimen was measured by using vernier calipers. The mean sample thickness was 1.147 mm, SD 0.47, SE 0.02.

Aluminium strips, approximately 0.5mm thick and 2mm wide, were folded in half and attached to (cyanoacrylate adhesive) and completely around both ends of the wall and sole material either side of the notched white line region. This gave a site of attachment for the clamps of the test machine with sufficient grip without slipping. To ensure 100% hydration before final testing, the prepared samples were returned to de-mineralised water for a further 24hrs at room temperature.

Test machine and tensile strength sample testing

A Davenport-Nene T10 test frame (Davenport-Nene Ltd, Wellingborough, UK) was used to perform tensile strength tests. The machine was fitted with a 260 Newton capacity load cell and tensile test grips. Tests were conducted to failure at a test speed of 10 mm min^{-1}. The peak load during the test, and final load (when the white line failed completely) were divided by the cross-sectional area of the waisted portion of the test specimen to give the peak stress of the white line.

Data analysis for tensile strength testing

All samples were treated and tested following identical protocol regardless of the sample date and supplement. Data were entered in a database using Microsoft Access for Windows '95, (Version 7, 1989-1995, Microsoft Corp.) and were analysed after all the mechanical tests had been completed. The samples that failed before the test began were recorded as missing data.

The data were checked for normal distribution using Minitab 10-5 (Minitab Inc.). Comparisons were made using two sample *t*-tests between biotin supplemented and unsupplemented cows, left and right hind feet, lateral and medial claws and zones 2 and 3 of the peak failure and end failure rate of all samples. Differences in white line properties between sample dates were analysed using analysis of variance (ANOVA).

This was followed by multivariate analysis using general linear models to incorporate the effects of all of the independent variables using Minitab 10-5 (Minitab Inc.).

Vickers microhardness testing

Two regions of the dairy cow foot were selected for testing Vickers microhardness (described later); zone 2 of the sole of the left and right hind medial claw of all of the remaining ten cows on day 187 (final sample, five from each supplementation), a total of twenty samples. The wall material was not selected for testing as it takes approximately 15 months before complete growth to the bearing surface of the wall horn following the start of biotin supplementation.

Sample preparation

Horn samples were prepared by cutting a strip of approximately 3mm x 5mm of horn using cutter A (Appendix V). These were taken from the horn that remained from the existing horn samples. The specimens were glued to 30 mm squares of perspex using cyanoacrylate adhesive. All specimens were at least eight times thicker than the expected indentation depth to remove the possible influence of stress-field disruption, as suggested by Vincent (1992).

The test surface was polished using 'wet and dry' abrasive paper starting initially with 600 grit rate, followed by 1200 grit rate paper. Final polishing was carried out using a small buffing wheel and 10 micron diamond cutting paste.

Specimens were equilibriated with conditions in the laboratory as wet specimens proved too soft (100% hydration) for measurements to be made using Vickers microhardness. Moisture content was quantified by weighing at testing and after drying.

Test machine and sample testing

The test used for this keratinised material was a Vickers microhardness test using a micro-indenter, which pressed a minute diamond pyramid into the hoof material. A test load of 20g was used.

For each microhardness test the following timings were used to standardise test conditions. Indenter descent time was approximately 10 seconds after which the indenter was allowed to dwell on the specimen for 10 seconds and then removed. A further 45 seconds elapsed before the indentation was measured. The length of the diagonals of each indentation were measured using a calibrated graticule in the objective of the microhardness test machine microscope. Vickers microhardness (VHN) was calculated using the formula below:

VHN=(1854*P)/d^2

Where P is the load (grams) and d is the diagonal length in microns.

Ten indentations per sample were made at random points over the surface of each sample.

Data analysis for Vickers microhardness test

Data were entered into a database using Microsoft Access for Windows '95, (Version 7, 1989-1995, Microsoft Corp.) Two sample *t*-tests were carried out to analyse any difference between the supplemented and unsupplemented cows and the lateral and medial zones using Minitab 10-5 (Minitab Inc.).

RESULTS

Milk biotin concentration

The milk samples taken before the beginning of the study indicated that the cows had very similar levels of circulating biotin, no significant difference was found (P 0.60) (Table 5.1). After supplementation had started, the cows supplemented with biotin had greater concentrations of circulating biotin, mean 506-521 nmol/kg compared with 142-183 nmol/kg in the unsupplemented cows (P <0.001, Table 5.2 and 5.3). There was an increase in the milk biotin concentration of approximately 260% in the supplemented cows (Tables 5.1, 5.2 and 5.3). These figures confirm that the supplementation was successful.

Table 5.1: Mean biotin concentration of milk samples taken prior to the trial

Biotin	Mean	N	St Dev	SE	P
Yes	196.87	4	59.76	29.88	0.60
No	177.55	4	33.52	16.78	

(nmol/kg) CI = 95% confidence interval, *P significant

Table 5.2: Mean biotin concentration of milk samples taken at 2 weeks into the trial

Biotin	Mean	N	St Dev	SE	P
Yes	521.0	4	129.0	64	0.01*
No	142.3	4	20.8	10	

(nmol/kg) CI = 95% confidence interval, *P significant

Table 5.3: Mean biotin concentration of milk samples taken 14 weeks into the trial

Biotin	Mean	N	St Dev	SE	P
Yes	506.0	4	130	65	0.02*
No	183.1	4	22.1	11	

(nmol/kg) CI = 95% confidence interval, *P significant

There were two batches of feed delivered during the trial. The supplemented feed contained 19.30 mg/kg biotin, 0.7 mg/kg less than the 20 mg/kg required for the trial. The unsupplemented ration contained 0.234 mg/kg slightly less than the 0.3 mg/kg expected. The use of the rolls corresponded with the expected use and with the second batch of feed delivery date estimated.

There was one lame cow during the trial which was identified at a sample collection visit.

Tensile strength

Peak yield tensile strength

Crude analysis identified that time at which the white line samples were collected had a significant ($P < 0.01$) effect on the mean tensile strength of the white line, measured in Megapascals (MPa), when all samples from supplemented and unsupplemented cows were compared (Table 5.4 and Figure 5.3). With the exception of sample 4 (day 147) the mean peak tensile strength increased from 3.79 MPa to 5.60 MPa. Sample 4 decreased in strength to just below, but similar levels to those observed at day or sample 0 (Table 5.4 and Figure 5.3).

Table 5.4: Crude associations of peak tensile strength (mega-pascals) for white line region by different variables over all sample periods

Variable	Number	Mean	St Dev	P	No cows
Unsupp	285	4.18	2.09	0.85	-
Biotin Suppl	286	4.21	2.16		-
Left hind	283	4.35	2.24	0.09	-
Right hind	288	4.04	1.99		-
Lateral claw	284	3.64	2.03	<0.01*	-
Medial claw	287	4.75	2.07		-
Zone 2	286	5.27	1.86	<0.01*	-
Zone 3	285	3.11	1.80		-
No wl damage	502	4.45	2.07	<0.01*	-
WL damage	69	2.35	1.46		-
Sample 0	111	3.79	1.79	<0.01*	14
Sample 1	109	3.46	1.89		14
Sample 2	112	4.19	2.02		14
Sample 3	80	4.94	1.99		10
Sample 4	80	3.63	1.78		10
Sample 5	79	5.60	2.53		10

P* Significant, P - *t*-test, P-sample ANOVA, WL-white line, Suppl-biotin supplemented, Unsuppl-unsupplemented.

Figure 5.3: Mean Peak tensile strength (MPa) by sample time

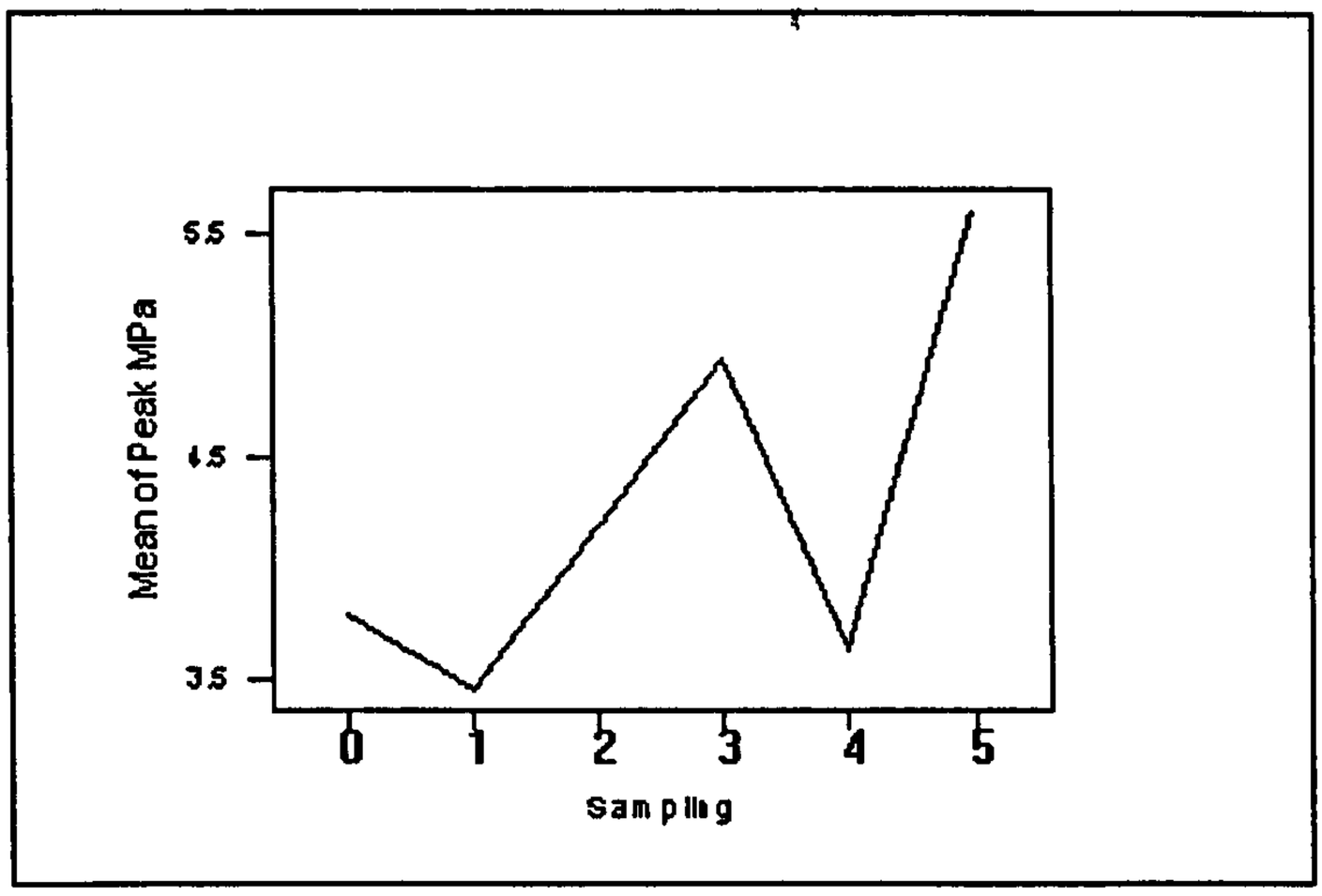
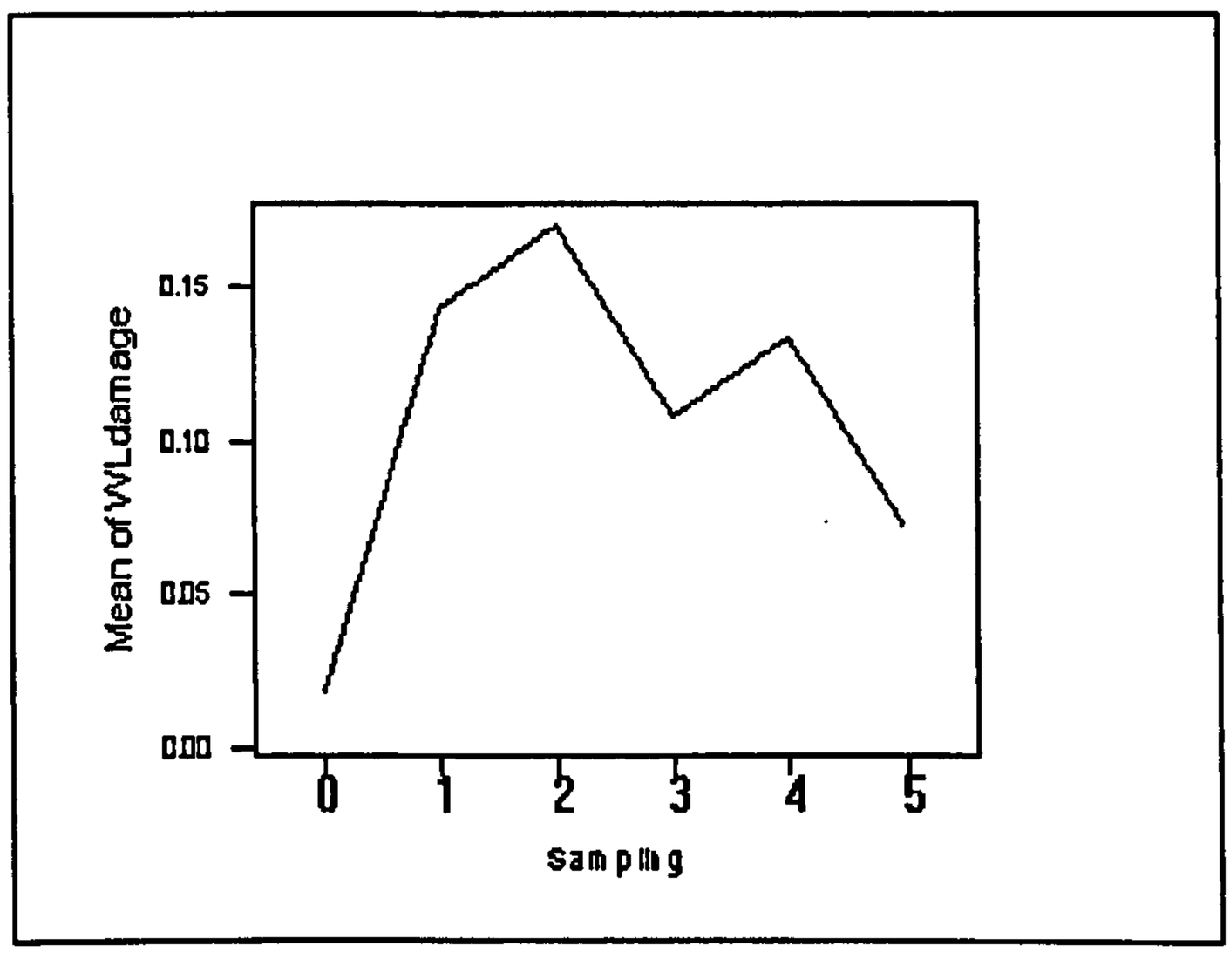


Figure 5.4: Mean amount of damaged white line material by sample time



Sample 0 - day 0, April/May 2000; Sample 1 - day 53, July 2000; Sample 2 - day 90, August 2000; Sample 3 - day 117, September 2000; Sample 4 - day 147, October, 2000; Sample 5 - day 187, November 2000; WL - white line lesion.

A significant difference ($P < 0.01$) in peak tensile strength was found in the white line between the zones tested (Table 5.4). Zone 2 (5.27 MPa) had a significantly higher tensile strength than zone 3 (3.11 MPa).

The peak strength of the white line samples that were grossly damaged (2.35 MPa) at testing (Table 5.4) were significantly ($P < 0.01$) weaker than those identified as normal (4.45 MPa).

A significant difference of peak tensile strength was also found in the white line between the lateral and medial claws ($P < 0.01$) (Table 5.4). The medial claw (4.75 MPa) was significantly stronger in testing than the lateral claw (3.64 MPa).

Biotin supplementation did not have a significant effect on peak tensile strength of the white line (Table 5.4 and 5.5). No significant difference in peak tensile strength of the white line was observed between the right (4.04 MPa) and left (4.35MPa) hind feet (P 0.09) (Table 5.4).

Data were split by sample collection day. The same pattern of increased mean peak tensile strength appeared over time with the exception of sample 1 (day 53) and 4 (day 147). Also, biotin supplemented cows had very slightly elevated white line tensile strength compared to the unsupplemented cows in all samples except for sample 1 (day 53) and 4 (day 147) (Table 5.5).

The different zones and lateral and medial claw remained significant for all samples 0 to 5. The mean strength increased with each sample period (4.41MPa sample 0, to 7.02 MPa sample 5) with the exception of sample 4 (4.67 MPa). Zone 3 tensile strength fluctuated over the sample dates (Table 5.5).

Table 5.5: Peak tensile strength (mega-pascals) for white line region by different variables, by sample collection date (t test)

	Sample 0		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
Variable	No	Mean	No	Mean	No	Mean	No	Mean	No	Mean	No	Mean
Unsupp	56	3.65 ±1.58	54	3.60 ±1.99	56	4.08 ±2.07	40	4.89 ±1.92	40	3.82 ±1.93	39	5.51 ±2.49
Suppl	55	3.94 ±1.99	55	3.31 ±1.78	56	4.30 ±1.99	40	4.99 ±2.08	40	3.44 ±1.62	40	5.70 ±2.61
P		0.39		0.43		0.58		0.83		0.35		0.75
Left hind	55	3.81 ±2.01	53	3.66 ±1.75	56	4.04 ±2.10	40	5.14 ±2.28	40	3.85 ±1.68	39	6.19 ±2.67
Right hind	56	3.77 ±1.57	56	3.27 ±2.00	56	4.34 ±1.96	40	4.74 ±1.66	40	3.41 ±1.87	40	5.04 ±2.29
P		0.90		0.29		0.43		0.37		0.27		0.04*
Lateral claw	56	3.24 ±1.63	53	3.10 ±1.71	56	3.62 ±1.96	40	4.32 ±2.11	40	3.19 ±1.77	39	4.72 ±2.62
Medial claw	55	4.35 ±1.79	56	3.80 ±1.99	56	4.76 ±1.94	40	5.56 ±1.66	40	4.07 ±1.70	40	6.47 ±2.15
P		<0.01*		0.05		<0.01*		<0.01*		0.02*		<0.01*
Zone 2	55	4.41 ±1.75	55	4.50 ±1.84	56	5.50 ±1.33	40	6.05 ±1.15	40	4.67 ±1.51	40	7.02 ±2.02
Zone 3	56	3.18 ±1.63	54	2.40 ±1.24	56	2.89 ±1.74	40	3.83 ±2.04	40	2.59 ±1.39	39	4.15 ±2.17
P		<0.01*		<0.01*		<0.01*		<0.01*		<0.01*		<0.01*
No wl damage	109	3.80 ±1.80	95	3.62 ±1.92	93	4.61 ±1.87	68	5.23 ±1.83	65	4.09 ±1.60	72	5.90 ±2.46
WL damage	2	2.85 ±0.32	14	2.38 ±1.19	19	2.15 ±1.46	12	3.31 ±2.14	15	1.65 ±1.07	7	2.59 ±0.64
P		0.08		<0.01*		<0.01*		<0.01*		<0.01*		<0.01*

P* Significant difference,Mean value ± SD, WL-white line, Med-medial claw, Lat-lateral claw, Suppl-biotin supplemented, Unsuppl-unsupplemented.

The medial claws had a significantly greater tensile strength than the lateral claws throughout the study. Again the strength of both claws increased with time (medial claw mean 3.80MPa to 6.47 MPa and lateral claw 3.10 MPa to 4.72MPa)

except for a depletion at sample 1 (day 53) and 4 (day 147) for the medial claw and a depletion in value in sample 1 for the lateral claw (Table 5.5).

A significant difference ($P<0.01$) was observed in the peak tensile strength of the white line samples that were classified damaged as opposed to those classified as undamaged (Table 5.5) in all individual sample periods except for day 0 which contained only 2 samples that were classified as damaged. When the damaged white line data were plotted (Figure 5.5) it corresponds in opposition to the pattern for peak tensile strength plotted by the sample dates.

The mean peak tensile strength values for the left and right hind feet (Table 5.5) increased with each sample, with the exception of sample 1 (day 53) and 4 (day147) with a range from approximately 3.27 MPa to 5.14 MPa in sample 0 to 4 which had similar values in both feet. By sample 5 (day 187) the left hind foot was significantly stronger (6.19 MPa) than the right hind foot (5.04 MPa) (P 0.04).

To investigate the effects of the variables combined, ANOVA using the general linear model was carried out. Table 5.6 shows pooled data. Lateral and medial claw, zone, sample date and white line damaged material remained highly significant in the analysis ($P<0.01$). The left hind or right hind foot was also significant (P 0.02). White line lesion was also incorporated into Table 5.6 and was not significant.

Table 5.6: General linear model for peak tensile strength (mega-pascals) of the white line for combined sample days

Variable	D F	Adj SS	F	P
Suppl/Unsuppl	1	0.18	0.07	0.79
WL lesion	1	0.01	0.00	0.95
WL damage	1	43.12	17.74	<0.01*
Left/right hind	1	14.13	5.81	0.02*
Lat/Med claw	1	110.41	45.43	<0.01*
Zones 2/3	1	535.91	220.50	<0.01*
Sample	5	301.71	24.83	<0.01*

P* Significant, WL -White line, Med-medial claw, Lat-lateral claw, Suppl-biotin supplemented, Unsuppl-unsupplemented.

Models were run to analyse the individual sample dates and can be observed in Appendix V. Findings were similar to the pooled data model. Although, only zone of the claw tensile strength remained significant throughout all sample dates.

After the data for the damaged white line samples were removed, the significant difference for peak white line tensile strength originally observed in certain variables became more significant in (Table 5.7).

Table 5.7: General linear model for peak tensile strength (mega-pascals) of the white line overall, with white line damaged data removed

Variable	D F	Adj SS	F	P
Suppl/Unsuppl	1	0.65	0.25	0.62
Left/right hind	1	15.51	6.01	0.01*
Lat/Med claw	1	106.98	41.43	<0.001*
Zones 2/3	1	481.67	186.52	<0.001*
Sample	1	294.09	22.78	<0.001*

P* Significant difference, WL-white line, Med-medial claw, Lat-lateral claw, Suppl-biotin supplemented, Unsuppl-unsupplemented.

Breaking tensile strength

The final yield or breaking strength of the white line was recorded and analysed in the same way as the peak tensile strength.

Table 5.8 shows all of the sample data pooled. The medial claw (1.67 MPa) had a significantly greater breaking strength than the lateral claw (1.15 MPa) (P <0.01). Zone 2 (1.88 MPa) also had a significantly higher breaking strength than zone 3 (0.94 MPa) (P <0.01). The grossly damaged white line material (0.77 MPa) had a significantly lower breaking strength than apparently normal tissue (1.50 MPa) (P<0.01). Sample date was also significant to the breaking strength (P 0.002).

Table 5.8: Crude comparisons of the final yield tensile strength (mega-pascals) for white line region by different variables over all sample periods

Variable	Number	Mean	St Dev	P
Unsupp	285	1.46	1.57	0.43
Suppl	286	1.36	1.71	
Left hind	283	1.54	1.78	0.06
Right hind	288	1.28	1.48	
Lateral claw	284	1.15	1.40	<0.01*
Medial claw	287	1.67	1.82	
Zone 2	286	1.88	1.83	<0.01*
Zone 3	285	0.94	1.26	
No wl damage	502	1.50	1.70	<0.01*
WL damage	69	0.77	0.87	
Sample 0	111	1.41	1.64	0.002*
Sample 1	109	0.97	1.11	
Sample 2	112	1.51	1.60	
Sample 3	80	1.74	1.71	
Sample 4	80	1.12	1.42	
Sample 5	79	1.82	2.21	

P* Significant difference, P t-test result, P sample ANOVA result, WL-white line, Suppl-biotin supplemented, Unsuppl-unsupplemented.

In Table 5.9 zone of the claw remained significantly different throughout all sample periods. Zone 2 was consistently stronger than zone 3 white line. The difference between the lateral and medial claws was only significant on sample 0 (day 0, P <0.01), 2 (day 90, P 0.04) and 3 (day 117, P 0.03). Although the medial claw was always stronger than the lateral claw.

The white line damaged material (Table 5.9) was stronger than the normal material in sample 1-5. In sample 0 the mean of the damaged white line material was

stronger than the normal horn but not significantly. Significant differences were observed in sample 2 (day 90, P <0.01), 4 (day147, P <0.01) and 5 (Day 187, P <0.01).

A significant difference between left and right hind white line strength was only found at day 0 (Table 5.9), the left had a greater breaking strength than the right (P <0.01). The other sample dates did not have a consistent pattern. Biotin supplementation did not have a significant effect on white line breaking strength and there were no distinctive patterns.

The results were analysed further in a general linear model (Table 5.10). Left and right hind (P 0.04), lateral or medial claw (P<0.01), zone 2 or 3 (P <0.01) and the sample time (P <0.01) all remained significant in the breaking tensile strength of the white line in pooled data analysis.

Table 5.9: Breaking tensile strength (mega-pascals) for white line region by different variables, by samples (t test)

	Sample 0		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
Variable	No	Mean	No	Mean	No	Mean	No	Mean	No	Mean	No	Mean
Unsupp	56	1.44 ±1.54	54	0.99 ±1.15	56	1.60 ±1.76	40	1.64 ±1.80	40	1.00 ±1.39	39	1.70 ±2.09
Suppl	55	1.38 ±1.75	55	0.95 ±1.07	56	1.43 ±1.43	40	1.84 ±1.63	40	1.25 ±1.46	40	1.94 ±2.34
P		0.83		0.86		0.58		0.61		0.44		0.63
Left hind	55	1.75 ±1.91	53	0.96 ±0.95	56	1.50 ±1.75	40	2.00 ±1.87	40	1.22 ±1.51	39	1.98 ±2.40
Right hind	56	1.08 ±1.26	56	0.98 ±1.25	56	1.53 ±1.45	40	1.49 ±1.50	40	1.03 ±1.35	40	1.67 ±2.03
P		0.03*		0.90		0.94		0.19		0.56		0.53
Lateral claw	56	1.09 ±1.36	53	0.90 ±1.02	56	1.21 ±141	40	1.33 ±1.59	40	1.06 ±1.28	39	1.41 ±1.74
Medial claw	55	1.74 ±1.83	56	1.04 ±1.19	56	1.82 ±1.73	40	2.16 ±1.74	40	1.19 ±1.57	40	2.23 ±2.54
P		<0.01*		0.53		0.04*		0.03*		0.70		0.10
Zone 2	55	1.78 ±1.75	55	1.27 ±1.27	56	2.13 ±1.84	40	2.22 ±1.86	40	1.67 ±1.71	40	2.38 ±2.42
Zone 3	56	1.05 ±1.44	54	0.67 ±0.82	56	0.90 ±1.00	40	1.27 ±1.40	40	0.57 ±0.75	39	1.25 ±1.83
P		0.02*		<0.01*		<0.01*		0.01*		<0.01*		0.02*
No wl damage	109	1.39 ±1.65	95	0.99 ±1.14	93	1.68 ±1.70	68	1.86 ±1.73	65	1.31 ±1.52	72	1.93 ±2.28
WL damage	2	2.33 ±0.42	14	0.85 ±0.87	19	0.72 ±0.50	12	1.09 ±1.46	15	0.31 ±0.28	7	0.76 ±0.69
P		0.22		0.60		<0.01*		0.12		<0.01*		<0.01*

P* Significant difference, Mean value ± SD, WL-white line, Suppl-biotin supplemented, Unsuppl-unsupplemented.

Table 5.10: General linear model for breaking tensile strength (mega-pascals) of the white line for combined sample dates

Variable	D F	Adj SS	F	P
Suppl/Unsuppl	1	1.92	0.83	0.36
WL lesion	1	1.51	0.65	0.42
WL damage	1	0.90	0.38	0.54
Left/right hind	1	9.62	4.13	0.04*
Lat/Med claw	1	31.24	13.42	<0.01*
Zones 2/3	1	113.72	48.84	<0.01*
Sample	5	51.01	4.38	<0.01*

P* Significant difference, WL-white line, Med-medial claw, Lat-lateral claw, Suppl-biotin supplemented, Unsuppl-unsupplemented.

Vickers hardness

Sole horn of the left hind lateral claw zone 2 and medial claw of zone 2 were tested for micro-hardness. The moisture content was calculated by recording the sample weight before testing and after drying. Biotin supplemented cows: left hind lateral claw 55% moisture, left hind medial claw 17.6% moisture. For the cows not supplemented with biotin the moisture content was: left hind lateral claw 22.5% moisture, left hind medial claw 49.1% moisture. After analysis using chi square the biotin supplemented lateral claw zone 2 had significantly higher (Yates corrected <0.001) moisture than the unsupplemented lateral claw zone 2. The unsupplemented medial claw zone 2 had significantly higher (Yates corrected P <0.001) moisture than the unsupplemented medial claw zone 2. Biotin supplementation did not have a significant effect on horn moisture content overall.

No significant effect of biotin supplementation on Vickers micro-hardness was found. Using t-test analysis (Table 5.11) the mean values were almost identical for both biotin supplemented and unsupplemented cows, also supported by a P value that was almost equal to 1, although the standard deviation from the mean was slightly larger for cows supplemented with biotin.

Table 5.11: Vickers hardness (Kg mm^{-2}) for sole horn by supplementation

Variable	Number	Mean	SD	P	DF
Biotin Supplemented	10	16.05	2.09	0.99	15
Unsupplemented	10	16.06	1.34		

P* Significant difference

No significant difference was found in hardness of the sole horn between the two different claws tested. A slightly higher mean hardness rating in the medial claw was found opposed to the lateral claw but the difference was not large enough to be significant (Table 5.12).

Table 5.12: Vickers hardness (Kg mm^{-2}) of sole horn by lateral and medial claw

Variable	Number	Mean	SD	P	DF
Lateral	10	15.56	1.36	0.21	16
Medial	10	16.55	1.94		

P* Significant

Table 5.13: Vickers Hardness (Kg mm^{-2}) ANOVA (general linear model) of sole horn

Variable	D F	Adj SS	F	P
Suppl/Unsuppl	1	0.001	0.00	0.99
Lat/Med claw	1	4.902	1.65	0.22

P* Significant difference

The data were fitted into a general linear model for the combined effect of the variables (Table 5.13). No significant effect of biotin supplementation was observed and no difference between the test sites were observed.

DISCUSSION

After a lag period between the first milk sample and the start of supplementation, because of confusion between the supplementing feeds and the cows which were to receive the different rations, (rectified by re-starting the study) supplementation was successful and a significant difference was observed between the biotin supplemented and unsupplemented cows. The cutters used in the study were successful in reducing the large variability that may have been encountered if the samples had been prepared by hand.

There was a significant difference between the peak tensile strength of the white line in the medial and lateral claws. The medial had significantly stronger white line than the lateral claws ($P < 0.01$). This was consistent when combined with other variables in the model overall.

Previous studies have indicated a difference between lateral and medial claws in structure (length, width, growth and wear), function and pathology (abnormal claw shape cause or effect of uneven weight distribution and potentially lesions) (Russell *et al* 1982) and others have not seen a difference (Andersson and Lundstrom 1981; Hahn *et al* 1984; Vermunt 1990; Tranter and Morris 1992; Budras *et al* 1996; Vermunt and Greenough 1996b). Pathological studies have also found differences in the morphology and internal aspects of the medial and lateral claws, so the forces are therefore different in weight bearing (Ossent *et al*, 1987, Toussaint Raven 1973). Baggott *et al* (1988) only observed a difference in claws in lame cows, where the lateral claw had a decreased hardness and an increased water, ash and magnesium content than the medial claw. Changes in lateral claws may be as a result of lesion occurrence or the difference encountered as a result of, for example, excessive loading

(Baggott *et al* 1988).

The peak tensile strength of the white line in different zones of the claw was highly significant in this study. Zone 3, which is the most caudal end of the white line on the abaxial border, was significantly weaker than the white line in zone 2 (Figure 5.1). Budras *et al* (1996) found that the abaxial region had the highest horn production of incompletely, potentially lower quality, keratinised horn. This is considered to be the most vulnerable site along with the axial termination and the junction between the coronary horn and white line.

In addition, zone 3 of the claw may undergo greater stress and strain and expansion (Baggott and Russell 1981) than zone 2 in locomotion as a result of loading in the stance phase, as previously detailed, which transfers from tensile forces into weight in the sole and heel border at zone 3 (Warzecha 1993; Kempson and Logue 1993). It is therefore, susceptible to damage and crack propagation (Bertram and Gosline 1986) and potentially lesions (Johnston 1990; Vermunt and Greenough 1990).

When these factors are considered, this may be the reason why most lameness is observed in the lateral claw (Tranter and Morris 1992; Midla *et al* 1998; Fitzgerald *et al* 2000; Hedges *et al* 2001) and more predominantly the abaxial border where most of the weight of the cow is loaded. (Vermunt and Greenough 1996a; Greenough *et al* 1997; Logue *et al* 1998a).

There were surprisingly differences in tensile strength that occurred in both biotin supplemented and unsupplemented animals in the trial. Given the rate of horn growth these differences were surprisingly rapid. These occurred either at the point of production 3 to 4 months earlier or at the point of wear. Possible hypothesis include stage of production, for example, sample collection 1 (0.05) corresponded with the month July, which was approximately peak lactation. It is proposed that this may be

the reason why there was a low mean peak tensile strength found for the lateral and medial claw white line at this time (3.10 MPa and 3.80 MPa respectively) and potentially why there was a marginal significance P = 0.05. As peak lactation approaches cows experience changes in energy and weight loss as energy mobilisation is used to meet the demands of milk production (Chamberlain and Wilkinson 1996). October or sample 4 (P=0.02) was a period of management transition and graphically (Figure 5.3 and 5.4) with an increased observation of white line damage in the white line sample material and a significant reduction in mean peak tensile strength.

Because of the significant reduction in tensile strength at sample collection 4 and the dramatic increase a month later, the damaged white line sample results were removed from the analysis to ensure no bias existed. The difference between the claws became more significant as a result (P<0.001).

The peak tensile strength for the visually damaged white line material was significantly weaker (P <0.01) than the white line material that did not display damage at inspection. When the data were analysed in the general linear model the damaged material peak tensile strength value were only significant in sample 4 (Appendix V) (P 0.02). This corresponds with the significant October depletion in peak tensile strength of the white line (Figure 5.3) and the peak observed in the white line damage curve (Figure 5.4) which have been discussed previously.

A significant difference in the peak tensile strength of the left and right hind feet was identified in the model (P 0.02). This was unexpected and a possible explanation may be in the day to day milking routine of the cows. After milking the cows always left the parlour by sharply turning left. Fitzgerald *et al* (2000) also found a higher damage score in the claws of the right hind foot than the left hind. Each contact made between the ground and the claw has been reported to change or

remodels the claw parameters (Vermunt and Greenough 1996b) and slipping is also reported to affect the conformation of the hooves (Phillips et a 1998). However, it may be that this process of turning on the left hind foot placed greater tensile, shearing and abnormal forces on that limb.

A few studies have found a significant influence of biotin supplementation on claw hardness and tensile strength but they have predominantly been carried out in the horse and pig (Kempson *et al* 1989; Zenker *et al* 1995).

Supplementation of biotin at 20mg per day in the dairy cow has been reported to increase horn hardness, particularly in the lateral wall (Distl and Schmid 1994), reduce certain lesions (Distl and Schmid 1994; Midla 1997; Hedges *et al* 2001), improve quality of horn (Hendry *et al* 1997; Vermunt and Greenough 1995b; Schmid and Geyer 1994; Kempson and Logue 1993) and improve tensile strength of the coronary horn (Schmid and Geyer 1994). So it was concluded that biotin supplementation may improve the tensile strength of the white line.

Schmid and Geyer (1994) studied a group of biotin supplemented cows without comparative unsupplemented animals. A significant increase in tensile strength was found after five months, quality increased which was observed by macroscopical and histological examination, although horn quality had already improved in the 3rd month of the study before the start of supplementation with biotin. This pattern of increased tensile strength was observed over the length of the current study (Figure 5.3), with the exception of sample 4, although the increase occurred in both the supplemented and unsupplemented cows, which emphasises the importance of having a comparative or control group.

The average values found for different white line sections in the study by Budras *et al* (1996) (5.1 N/mm^2 for the middle part of the zona alba (cap-horn the

main portion), 6.9 N/mm^2 for the inner part (terminal horn the main portion)) were similar to but slightly higher than the measurements found for the complete structure in the present study, which ranged from 3.63 N/mm^2 to 5.60 N/mm^2, depending on the time of year the sample was collected. The samples were taken from the weight-bearing surface of the horn of live animals and from the evidence seen in previous studies this is the region of 'oldest' horn material and would therefore be a structure most likely to contain microcracks and damage (Zenker *et al* 1995). Samples from previous studies have often been extracted from feet obtained at an abattoir to study the tensile strength of bovine hoof horn.

The mechanical properties of dairy cow hoof horn can alter in response to previously discussed factors such as hormonal changes and to hydration or dehydration. Martig *et al* (1980) identified a higher water content and softness in the hind claws opposed to the front claws and in drying, elasticity, a major function of the white line, may decline leaving the structure weaker (Budras *et al* 1996) although the moisture content does not decline below 25% *in vivo* (Baille *et al* 2000). So to standardise the samples of white line material tested for tensile strength, they were tested at 100% hydration by soaking the horn in de-mineralised water to mimic the true moisture uptake that would occur *in vivo*. Other methods have included increasing humidity to impose increased hydration in the cows hoof (Baillie *et al* 2000).

The sole horn samples that were prepared for Vickers hardness tests were also going to be tested at 100% hydration. However, the material was too soft and measurement through indentation was impossible. The samples were allowed to dry overnight and were all tested at the same time the following day. The sample weights were taken at testing to assess the percentage hydration. There were significant

differences (Yates corrected <0.001) between hydration in the material when compared by supplementation. The sole horn of the lateral claw of biotin supplemented and the medial claw of unsupplemented cows retained greater moisture than their opposing supplement claws, respectively. Therefore variable effects were observed in horn moisture retention. The reason for this cannot be explained. Schmid and Geyer (1994) found that moisture content and growth was not associated with dietary biotin. Baillie *et al* (2000) compared some of the hydration levels that had been observed in other studies for cows which include 32.5% (Baggott *et al* 1988), 23.1% and 28.8%, which are comparable to the moisture content in the horn at testing in this study with an average of 36% moisture.

Increased moisture content and hardness are inversely related to biomechanical properties or keratin rigidity (Leach and Zoerb 1983; Douglas *et al* 1996). Hardness and moisture content in the coronary and sole horn showed a negative correlation in a study by Schmid and Geyer (1994). Indentation hardness can also be used as a measure of resistance to abrasive wear, increased hardness equals increased resistance to wear (Bonser 1996).

Baggott *et al* (1988) also did not find a difference in hardness as a result of biotin supplementation. The samples in the current study were restricted to the final sample period in November 2000 and the lateral and medial claws of the left hind limb. Comparative testing over time in the other feet, claws and zones would have been valuable alongside the white line tensile strength values obtained. No significant difference was observed between the sole horn of zone 2 of the lateral and medial claws on the left hind claw (P 0.22).

CONCLUSION

This study investigated the tensile strength of the white line and the micro-hardness of the sole adjacent to the white line of the bovine foot and the impact of biotin supplementation.

The significant findings were that the lateral claw white line had a lower tensile strength than the medial claw, and zone 2 white line tensile strength was higher than zone 3. These biomechanical findings are supported by previous studies on the nature of weight distribution and conformation qualities.

Since 90% of lameness is attributed to claw disorders (Lischer 1993 cited by Warzecha 1993), further investigations of the complete foot and all regions of the white line would provide vital information that may assist in reducing lameness occurrence.

CHAPTER SIX

GENERAL CONCLUSIONS AND FUTURE RESEARCH

The aim of this research project was to investigate the effects of biotin supplementation on the overall incidence of lameness on five commercial dairy farms over eighteen months and its impact on individual lesions. This lead to a further small study on the effects of biotin on biomechanical claw properties of dairy cows.

The main finding of the trial was a significant reduction of white line lesion lameness by approximately half (hazard ratio 0.59), in cows supplemented with 20mg biotin. Biotin had a consistent effect on all farms and all lactations, which highlighted the strength of the likelihood of generalisability. So it is likely that dietary biotin supplement may help to reduce white line lesion lameness on farms with a high incidence, in conjunction with control measures preventing other potential farm level risk factors.

The overall incidence of lameness, which included limb lameness unassociated with the claw, was 68.9 cases per 100 cows per year which exceeds previous reports of 55 new cases per 100 cows per year (Clarkson *et al* 1996).

Biomechanical testing of the white line identified a reduced tensile strength in the lateral claw compared to the medial claw, and in zone 3 of the claw compared with zone 2.

The highest proportion of white line lesions, in previous studies (Logue *et al* 1998) as well as the present study, are reported to be located in the lateral hind claw at the site of zone 3 where the white line tensile strength was found to be weaker in the

present study. It is also the abaxial termination of the white line (Budras *et al* 1996), which may explain the predilection for damage at this particular site.

A significant difference was not found between the tensile strength of the white line, or micro-hardness of the sole directly adjacent to the white line as a result of biotin supplementation. The reason for this is not known. There may have been small undetectable differences that would have been identified if the study group had been larger. Also, white line lesion lameness increases with increasing lactation number so the effect of biotin may have been more obvious in older cows (Offer, McNulty and Logue 2000). Because of the small sample size, only very large effects would have been detected and so, whilst the study identified interesting and useful information about the variations in tensile strength along the white line, the effects of biotin are inconclusive.

Due to the significant economic and welfare implications of lameness, it is surprising that very little biomechanical research has been carried out on bovine horn to date.

Further and more in-depth investigation into micro-hardness testing, which was restricted to one zone of one limb in the present study, tensile strength testing and other objective measures of claw biomechanics and the mechanical properties of the environment, would improve our understanding of the properties of the bovine claw. This combined with early observations of lesion and lameness development together, could provide vital information to reduce and possibly prevent lameness, especially in later lactations.

REFERENCES

Abel HJ, DaCosta Gomez C (1997) Effect of varying roughage/concentrate-proportions on biotin balance in RUSITEC. July, 1997 (Research report, Hoffmann-La Roche Ltd.).

Alban L (1995) Lameness in Danish dairy cows: frequent and possible risk factors. Prev.Vet. Med. 22: 213-225.

Alban L, Lawson LG, Agger JF (1995) Foul in the foot (interdigital necrobacillosis) in Danish dairy cows: frequency and possible risk factors. Prev. Vet. Med. 24: 73-82.

Albarano T (1993) Der Einfluss der Umgebung auf die Zugfestigkeit und Harte des Klauenhorns von Rind und Schwein. MSc Thesis, University of Zurich.

Altman DG (1991) Practical statistics for medical research. Chapman and Hall/CRC, Edition 1.

Andersen PH, Jarlov N (1990) Investigation of the possible role of endotoxin, TXA2, PGI2 and PGE2 in experimentally induced rumen acidosis in cattle. Acta. Vet. Scand. 31: 27-38.

Andersson L, Lundstrom K (1981) The influence of breed, age, body weight and season on digital diseases and hoof size in dairy cows. Zbl. Vet. Med. A 28, 141-151.

Argaez-Rodriguez FJ, Hird DW, Hernandez de Anda J, Read DH, Rodriguez-Lainz A (1997) Papillomatous digital dermatitis on a commercial dairy farm in Mexicali, Mexico: Incidence and effect on reproduction and milk production. Prev. Vet. Med. 32: 275-286.

Arkins S (1981) Lameness in dairy cows. Irish Vet. J. June pp136-170.

Baggott DG, Bunch KJ, Gill KR (1988) Variations in some inorganic components and physical properties of claw keratin associated with claw disease in the British Friesian cow. Brit. Vet. J. 144: 534-542.

Baggott DG, Russell AM (1981) Lameness in cattle. Brit. Vet. J. 137: 113-132.

Baillie C, Southam C, Buxton A, Pavan P (2000) Structure and properties of bovine hoof horn. Advanced composites letters Vol. 9, No 2. 101-113.

Bargai U (1998) Digital dermatitis, interdigital dermatitis and heel erosion - Are these separate diseases? 10th Int. Symp. Disorders of the Ruminant Digit pp 265.

Bargai U, Mazrier H (2000) Epidemiological aspects of the sequelae of subclinical laminitis. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 143-148.

Batra TR, Hidiroglou M, Smith MW (1992) Effect of vitamin E on incidence of mastitis in diary cattle. Canad. J. Anim. Sc. 72: 287-297.

Berg JN, Franklin CL (2000) Interdigital phlegmon a.k.a. Interdigital necrobacillosis a.k.a. Acute foot rot of cattle: Considerations in etiology, diagnosis and treatment. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 27-30.

Bergsten C (1994) Haemorrhages of the sole horn of dairy cows as a retrospective indicator of laminitis: an epidemiological study. Acta. Vet. Scand. 35: 55-66.

Bergsten C, (1995) Digital disorders in dairy cattle and special reference to laminitis and heel erosion: The influence of housing, management and nutrition. Dissertation. Skara Sweden (Abstract).

Bergsten C, Frank B (1996) Sole haemorrhages in tied primiparous cows as an indicator of periparturient laminitis: effects of diet, flooring and season. Acta. Vet. Scand. 37: 383-394.

Bergsten C, Herlin AH (1996) Sole haemorrhages and heel horn erosion in dairy cows: the influence of housing system on their prevalence and severity. Acta. Vet. Scand. 37: (4) 395-408.

Bergsten C, Hultgren J, Manske T (1998) Claw traits and foot lesions in Swedish dairy cows in relation to trimming interval and housing system. A preliminary report. 10th Int. Symp. Disorders of the Ruminant Digit pp 46-48.

Berry RJ, Logue DN, Appleby MC, Waran NK, Offer JE (1998a) Relationship between hoof lesions and the behaviour of cubicle housed Holstein-Friesian cows. 10th Int. Symp. Disorders of the Ruminant Digit pp 56-57.

Berry, RJ, Waran NK, Appleby MC, Logue DN (1998b) Subclinical hoof lesions and their significance for lying down behaviour in housed dairy cows. 10th Int. Symp. Disorders of the Ruminant Digit pp 44-45

Bertram JEA, Gosline JM (1986) Fracture toughness design in horse hoof keratin. J. Exp. Biol. 125: 29-47.

Bertram JEA, Gosline JM (1987) Functional design of horse hoof keratin: The modulation of mechanical properties through hydration effects. J. Exp. Biol. 130, pp121-136.

Blowey RW (1993) Cattle lameness and hoofcare. Farming Press. Edition 1.

Blowey RW (1999) personal communication.

Blowey RW, Done SH, Cooley W (1994) Observations on the pathogenesis of digital dermatitis in cattle. Vet. Rec. 135: 115-117.

Blowey RW, Greenough PR (1998) Cattle lameness and hoofcare. Education programme proceedings, June 1998.

Blowey RW, Ossent P, Watson CL, Hedges VJ, Green LE, Packington AJ (2000) Possible distinction between sole ulcers and heel ulcers as a cause of bovine lameness. Vet. Rec. 147, 110-112.

Boettcher PJ, Dekkers JCM, Warnick LD, Wells SJ (1998) Genetic analysis of clinical lameness in dairy cattle. J. Dairy Sc. 81: 1148-1156.

Bohli E (1993) Die Normalwerte der Zugfestigkeit des Klauenhorns von Rind und Schwein. Msc Thesis, University of Zurich.

Bolliger C (1991) The equine hoof: morphological and histochemical findings. Dissertation, Uni. Zurich.

Bonomi A, Quarantelli A, Sabbioni A, Superchi P (1996) Dairy cattle ration integration with rumen protected biotin. Effects on production and reproductive efficiency (experimental contribution). Inst. Zootechnics. Fac. Vet. Med. Uni. Parma.

Bonser RHC (1996) Short communication – Comparative mechanics of bill, claw and feather keratin in the common starling sturnus vulgaris. J. Avian Biol. 27: 1.

Bonser RHC (1999) The young's modulus of ostrich claw keratin. J. Materials Sc. Letters (in press).

Bonser RHC, Witter MS (1993) Indentation hardness of the bill keratin of the European starling. The Condor 95: 736-738.

Brizzi A, Bergsten C, Blowey RW, Collick D, Greenough P, Logue DN, van Amstel S, Vermunt JJ (1998) Description and terminology for claw capsule lesions (Workshop report). 10th Int. Symp. Disorders of the Ruminant Digit pp 98-127.

Brooks PH, Simmins PH (1980) Influence of biotin supplementation on foot lesions in sows. Vet. Rec. (letter, Nov 1).

Brooks PH, Smith DA, Irwin VCR (1977) Biotin-supplementation of diets; the incidence of foot lesions, and the reproductive performance of sows. Vet. Rec. 101: 46-50.

Bryant KL, Kornegay ET, Knight JW, Webb KE Jr, Notter DR (1985b) Supplemental biotin for swine I. Influence on feedlot performance, plasma biotin and toe lesions in developing gilts. J. Anim. Sc. 60: (1) 136-144.

Bryant KL, Kornegay ET, Knight JW, Webb KE Jr, Notter DR (1985a) Supplemental biotin for swine II. Influence of supplementation to corn and wheat-based diets on reproductive performance and various biochemical criteria of sows during four parities. J. Anim. Sc. 60: (1) 145-153.

Bryant KL, Kornegay ET, Knight JW, Veit HP, Notter DR (1985c) Supplemental biotin for swine III. Influence of supplementation to corn and wheat based diets on the incidence and severity of toe lesions, hair and skin characteristics and structural soundness of sows housed in confinement during four parities. J. Anim. Sc. 60: (1) 154-162.

Buda S, Mulling C (2000) Innervation of the bovine hoof. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 100-101.

Budras KD, Geyer H, Maierl J, Mulling CKW (1998a) Anatomy and structure of hoof horn (Workshop report). 10th Int. Symp. Disorders of the Ruminant Digit pp 176-188.

Budras KD, Hochstetter T, Mulling C, Nattermann W (1997) Structure, function and horn quality in the bovine hoof: the influence of nutritional and environmental factors. July 18 1997 (Research manuscript, Hoffmann-La Roche Ltd.).

Budras KD, Hullinger RL, Sack WO (1989) Light and electron microscopy of keratinisation in the laminar epidermis of the equine hoof with reference to laminitis. Am J Vet Res 50:1150-1160.

Budras KD, Mulling C, Horowitz H (1996) Rate of keratinization of the wall segment of the hoof and its relation to width and structure of the zona alba (white line) with respect to claw disease in cattle. AJVR 57: (4) 444-455.

Budras KD, Schiel C, Mulling C (1998b) Horn tubules of the white line: an insufficient barrier against ascending bacterial invasion. Eq. Vet. Ed. 10: (2) 81-85.

Buffa EA, Van den Berg SS, Verstraete FJM, Swart NGN (1992) Effect of dietary biotin supplementation on equine hoof horn growth rate and hardness. Eq. Vet. J. 24 (6) 472-474.

Campbell J, Greenough PR, Petrie L (1996) The effect of biotin on sandcracks in beef cattle. 9th Int. Symp. Disorders of Ruminant Digit. 14-19 April.

Chamberlain AT, Wilkinson JM (1996) Feeding the Dairy Cow. Chalcombe publications, Edition1, pp128.

Chaplin SJ, Offer JE, Logue DN (1998) Metabolic stress and welfare in relation to development of lesions of the claw in first lactation Holstein-Friesian heifers. 10th Int. Symp. Disorders of the Ruminant Digit pp 58-59.

Chesterton RN, Pfeiffer DU, Morris RS, Tanner CM (1989) Environmental and behavioural factors affecting the prevalence of foot lameness in New Zealand dairy herds-a case-control study. NZ Vet. J. 37: 135-142.

Choi YS, McDaniel BT (1993) Heritabilities of measures of hooves and their relation to other traits of Holsteins. J. Dairy Sc. 76: 1989-1993.

Choquette-Levy L, Baril J, Levy M, St Pierre H (1985) A study of foot disease of dairy cattle in Quebec. Can. Vet. J. 26: 278-281.

Clackson DA, Ward WR (1991) Farm tracks, stockman's herding and lameness in dairy cattle. Vet. Rec. 129:511-512.

Clark AK, Rakes AH (1982) Effect of methionine hydroxy analog supplementation on dairy cattle hoof growth and composition. J. Dairy Sc. 65: 1493-1502.

Clarkson MJ, Downham DY, Faull WB, Hughes JW, Manson FJ, Merritt JB, Murray RD, Russell WB, Sutherst JE, Ward WR (1996) Incidence and prevalence of lameness in dairy cattle. Vet. Rec. 138: 563-567.

Colam-Ainsworth P, Lunn GA, Thomas RC, Eddy RG (1989) Behaviour of cows in cubicles and its possible relationship with laminitis in replacement dairy heifers. Vet. Rec. 125: 573-575.

Collett D (1994) Modeling survival data in medical research. Chapman and Hall London.

Collick DW, Ward WR, Dobson H (1989) Associations between types of lameness and fertility. Vet. Rec. 125: 103-106.

Comben N, Clark RJ, Sutherland DJB (1984) Clinical observations on the response of equine hoof defects to dietary supplementation with biotin. Vet. Rec. 115: 642-645.

Cooke BC, Brumby PE (1982) Biotin - a dairy herd feeding trial. Roche Vit. Symp. London pp 21-26.

Coulon J, D'Hour P, Garel JP, Petit M (1995) Long term effect of level and pattern of winter concentrate allocation in dairy cows. 2emes rencontres autor des recherches sur les ruminants Paris, France, 13-14 decembre. 211-214 (abstr.)

Coulon J, Pradel P, Cochard T, Poutrel B (1998) Effect of extreme walking conditions for dairy cows on milk yield, chemical composition and somatic cell count. J. Dairy Sc. 81 (Supp. 4) 994-1003.

Cox D R (1972) "Regression Models and Life Tables," J. of the Royal Stat. B34, pp187-220.

Da Costa Gomez C, Almasri M, Steinberg W, Abel HJ (1998) Effect of varying hay/barley-proportions on microbial biotin metabolism in the rumen simulating fermentor RUSITEC. Proc. Soc. Nutr. Physiol. 7: 30.

Dakshinamurti K, Chauhan J (1988) Regulation of biotin enzymes. Ann. Rev. Nutr. 8: 211-233.

Dean AD, Dean JA, Burton AH, Dicker RC (1991) Epi Info version 6.04 USD, Stone Mountain, Georgia.

De Vecchis L, Mortellaro CM (1998) Vertical fissure in dairy cows. 10th Int. Symp. Disorders of the Ruminant Digit pp 136-138.

Distl O (1998) Genetic improvement of foot and leg soundness in dairy cattle. 10th Int. Symp. Disorders of the Ruminant Digit pp 330.

Distl O, Huber M, Graf F, Krausslich H (1984) Claw measurements of young bulls at performance testing stations in Bavaria. Liv. Pod. Sc. 11, 587-598.

Distl O, Mair A (1990) Pedobarometric forces at the sole/floor face. Proceedings of 6th International Symposium of Diseases of the Ruminant Digit. pp143-162.

Distl O, Schmid D (1994) The influence of biotin supplementation on the conformation, hardness and health of claws of dairy cows. Originalien 9: 582-584, 597-588. (abstr).

Distl O (1995) Genetic improvement of traits of feet and legs as well as claw soundness in cattle. Zuchtungskunde 67: (6) 438-448.

Dopfer D (2000) Summary about research activities concerning (Papillomatous) digital dermatitis in cattle published or developed since 1998. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 21-26.

Dopfer D, Willemen M (1998) Standardisation of infectious claw diseases (Workshop report). 10th Int. Symp. Disorders of the Ruminant Digit pp 244-264.

Douglas JE, Biddick TL, Thomason JJ, Jofriet JC (1998) Stress/strain behaviour of the equine laminar junction. J. Exp. Biol. 201: 2287-2297.

Douglas JE, Mittal C, Thomason JJ, Jofriet JC (1996) The modulus of elasticity of equine hoof wall: implications for the mechanical function of the hoof. J. Exp. Biol. 199: 1829-1836.

Eddy RG, Scott CP (1980) Some observations on the incidence of lameness in dairy cattle in Somerset. Vet. Rec. 106: 140-144.

Elias PM (1981) Lipids and the epidermal permiability barrier. Arch. Derm. Res. 270, 95-117.

Enevoldsen C, Grohn YT, Thyson I (1991a) Heel erosion and other interdigital disorders in dairy cows: associations with season, cow characteristics, disease and production. J. Dairy Sc. 74: 1299-1309.

Enevoldsen C, Grohn YT, Thyson I (1991b) Sole ulcers in dairy cattle: Associations with season, cow characteristics, disease and production. J. Dairy Sc. 74: 1284-1298.

Erskine RJ, Bartlett PC, Herdt T, Gaston P (1997) Effects of parenteral administration of vitamin E on health of periparturient dairy cows. JAVMA 211: (4) .

Farm Animal Welfare Council (1997) Report on the welfare of dairy cattle, December 1997. FAWC, Government buildings, Hook rise south, Tolworth, Surbiton, Surrey.

Faull WB, Hughes JW, Clarkson MJ, Downham DY, Manson FJ, Merritt JB, Murray RD, Russell WB, Sutherst JE, Ward WR (1996) Epidemiology of lameness in dairy cattle: the influence of cubicles and indoor and outdoor walking surfaces. Vet. Rec. 139: 130-136.

Fitzgerald T, Norton BW, Elliott R, Podlich H, Svendsen OL (2000) The influence of long-term supplementation with biotin on the prevention of lameness in pasture fed dairy cows. J. Dairy Sc. 83: 338-344.

French (2000) Epidemiological methods in the investigation of bovine lameness. XIth Int. Symp. on Disorders of the Ruminant Digit and Int. Conf. on Bovine Lameness, pp18-20.

French NP, Wall R, Morgan KL (1994) Lamb tail docking: a controlled field study of the effects of tail amputation on health and productivity. Vet. Rec. 134: 463-467.

Frigg M, Hartmann D, StrauB OC (1994) Biotin kinetics in serum of cattle after intravenous and oral dosing. Int. J. of Vit. and Nutr. Res. 64:36-40.

Frigg M, StrauB OC, Hartmann D (1993) The bioavailability of supplemental biotin in cattle. Int. J. Vit. Nutr. Res. 63: (2) 122-128.

Fritsche A (1990) Biotin verandert das Zytokeratinmuster von kultivierten Keratinozyten. DVM Thesis, Faculty of Vet. Med. Uni. Zurich.

Fritsche A, Mathis GA, Althaus FR (1991) Pharmakologische wirkungen von biotin auf epidermiszellen. Schweiz. Arch. Tierheilk. 133: 277-283.

Galbraith H, Mengal MA, Scaife JR (1998) Effect of dietary methionine and biotin supplementation on growth and protein and amino acid composition of caprine hoof horn. 10th Int. Symp. Disorders of the Ruminant Digit pp 227-232.

Geyer H (1984) Histochemische Untersuchungen an der Klauenepidermis des Schweines. Zbl. Vet. Med. C. Anat. Hist. Embryol. 13, 120-140.

Green LE, Bernatau E, Morgan KL (1994) Problems and some solutions in the collection of data when investigating diseases of lambs in early lambing (housed) flocks. Prev. Vet. Med. 18: 275-285.

Green, L. E., Graham M, Morgan KL (1997) A preliminary trial to investigate the effect of iron dextran on the prevention of a non-regenerative anaemia in housed lambs. Vet. Rec. 140: 219-222.

Greenough PR (1990) Observations on bovine laminitis. In Prac. July 169-173.

Greenough PR, Vermunt JJ (1991) Evaluation of subclinical laminitis in a dairy herd and observations on associated nutritional and management factors. Vet. Rec. 128: 11-17.

Greenough PR, Weaver AD (1997) Lameness in cattle. W B Saunders co, Third Edition, pp 3-122.

Greer EB, Leibholz JM, Pickering DI, Macoun RE, Bryden WL (1991) Effect of supplementary biotin on the reproductive performance, body condition and foot health of sows on three farms. Aust. J. Agric Res. 42: 1013-1021.

Hahn MV, McDaniel BT, Wilk JC (1984) Description and evaluation of objective hoof measurements of diary cattle. J. Dairy Sc. 67, 229-236.

Harris DJ, Hibburt CD, Anserson GA, Younis PJ, Fitspatrick DH, Dunn AC, Parsons IW, McBeath NR (1988) The incidence, cost and factors associated with foot lameness in dairy cattle in south-western Victoria. Aus. Vet. J. 65: (6) 171-176.

Hayward A F (1976) Ingestion of colloid in a keratinised epithelium and its localisation in membrane-coating granules. J. Anat. 121, 313-321.

Hedges VJ, Blowey RW, Packington AJ, O'Callaghan CJ, Green LE (2001) A longitudinal field trial of the effect of biotin on lameness in dairy cows. J. Dairy Sc. (in press).

Hendry KAK, MacCallum AJ, Knight CH, Wilde CJ (1997) Laminitis in the dairy cow: a cell biological approach. J. Dairy Res. 64: 475-486.

Higuchi H, Nagahata H (2000) Relationship between serum biotin concentration and moisture content of the sole horn in cows with clinical laminitis and in cows with sound hooves. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 295-297.

Hirschberg RM (2000) Microvasculature and microcirculation of the healthy and diseased bovine claw. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 97-99.

Hirst WM, French NP, Murray RD, Ward WR (2000) The importance of first lactation lameness as a risk factor for subsequent lameness: A mixed effects event time approach. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 149-151.

Hochstetter T (1998) Die Hornqualitat der Rinderklaue unter Einfluss einer Biotinsupplementierung. Dissertation, University Berlin.

Hochstetter T, Mulling C, Budras KD (1996) Effects of biotin on the horn quality of the bovine hoof. (Hoffmann-La Roche, Research report).

Holah DE, Evans KM, Pearson GR, Tarlton JF, Webster AJF (2000) The histology and histopathology of the support structures in the laminated region of the bovine hoof in maiden heifers and around the time of first calving, (poster). XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 109-111.

Hosmer DW, Lemeshow S (1999) Applied survival analysis; regression modeling of time to event data. Wiley Inter-science, Edition 1.

Hunkeler A, Lischer CJ, Geyer H, Ossent P (1996) The effect of biotin in the treatment of uncomplicated claw lesions with exposed corium in dairy cows part II: the healing process in supplemented animals. 9th Int. Symp. Disorders of the ruminant digit, Jerusalem.

Hurstel O (1982) Supplementation of calf milk replacer diets with biotin. Roche Vit. Symp. London, pp 5-11.

Johnston AM (1990) Studies of hoof horn. Proc. VIth Int. Symp. on Diseases of the Ruminant Digit. pp 76-79.

Johnston AM, Penny RHC (1989) Rate of claw horn growth and wear in biotin supplemented and non-supplemented pigs. Vet. Rec. 125: 130-132.

Josseck H, Zenker W, Geyer H (1995) Hoof horn abnormalities in Lipizzaner horses and the effect of dietary biotin on macroscopic aspects of hoof horn quality. EVJ 27 (3) 175-182.

Kempson SA (1987) Scanning electron microscope observations of hoof horn from horses with brittle feet. Vet. Rec. 120: 568-570.

Kempson SA (1990) Ultrastructural observation on the response of equine hoof defects to dietary supplementation with farrier's formula. Vet. Rec. 127: 494-498.

Kempson SA, Currie RJW, Johnston AM (1989) Influence of biotin supplementation on pig claw horn: a scanning electron microscopic study. Vet. Rec. 124: 37-40.

Kempson SA, Logue DN (1993) Ultrastructural observations of hoof horn from dairy cows: changes in the white line during the first lactation. Vet. Rec. 132: 524-527.

Kerr KL (1998) Affecting the incidence of lameness by altering the housing. 10th Int. Symp. on Lameness in Ruminants. pp 38-39.

Kirkwood BR (1988) Essentials of medical statistics. Blackwell Scientific Publications. Edition 1.

Klunter AM, Steinberg W, Schuep W (1993) Influence of biotin supplementation on the concentration of biotin in blood plasma and milk of dairy cows. (Hoffmann_la Roche, Research report).

Koller U, Lischer CJ, Geyer H, Ossent P, Schulze J, Auer JA (1998) The effect of biotin in the treatment on uncomplicated sole ulcers in cattle. A controlled study. 10th Int. Symp. Disorders of the Ruminant Digit pp 230-232.

Kopinski JS, Leibholz J, Bryden WL (1989) Biotin studies in pigs. 3. Biotin absorption and synthesis. Brit. J. Nutr. 62: 767-772.

Kornegay ET (1986) Biotin in swine production: a review. Liv. Prod. Sc. 14: 65-89.

Kossaibati MA, Esslemont RJ (1997) The costs of production diseases in dairy herds in England. Vet. J. 154: 41-51.

Kossaibati MA, Esslemont RJ (2000) The incidence of lameness in 50 dairy herds in England (poster). XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 160-162.

Kossaibati MA, Esslemont RJ, Watson C (1999) Understanding and tackling lameness in dairy herds. Booklet published by the University of Reading and National Milk Records PLC. UK.

Landeau L J, Barrett DJ, Batterman SC (1983) Mechanical properties of equine hooves. Am. J. Vet. Res. 44, 100-102.

Leach DH (1993) Structural changes in intercellular junctions during keratinisation of the stratum medium of the equine hoof wall. Acta. Anat. 147, 45-55.

Leach KA, Logue DN, Kempson SA, Offer JE, Ternent HE, Randall JM (1997) Claw lesions in dairy cattle: development of sole and white line haemorrhages during first lactation. Vet. J. 154: 215-225.

Leach KA, Logue DN, Randall JM, Kempson SA (1998) Claw lesions in dairy cattle: methods for assessment of sole and white line lesions. Vet. J. 155: 91-102.

Leach KA, Logue DN, Svoboda I, Offer JE, Middlemass CA (2000) Effect of diet fed to youngstock on lameness, heel erosion, lying time and properties of slurry. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 333-336.

Leach DH, Zoerb GC (1983) Mechanical properties of equine hoof wall tissue. Am. J. Vet. Res. 44: (11)

Leaver D (1990) Effects of feed changes around calving on cattle lameness. Proc. VIth Int. Symp. on Diseases of the Ruminant Digit. pp 102-108.

Le Fevre AM, Logue DN, Offer JE, McKendrick I, Gettinby G (2001) Correlations of measurements of subclinical claw horn lesions in dairy cattle. Vet. Rec. 148, 135-138.

Linden JE (1992) The role of biotin in improving the hoof condition of horses. Hoffmann-La Roche publication.

Lischer CJ, Hunkeler A, Geyer H, Ossent P (1996) The effect of biotin in the treatment of uncomplicated claw lesions with exposed corium in dairy cows: Part II: the healing process in supplemented animals. 9th Int. Symp. Disorders of the ruminant digit. April.

Lischer CJ, Ossent P (2000) Sole ulcers in dairy cattle - What's new about an old disease. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 46-48.

Lischer CJ, Ossent P, Raber M, Geyer H (2000) The significance of the suspensory mechanism of the third phalanx and it's fat bodies in the pathogenesis of sole ulcers in cattle. Part I: Macroscopic findings. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 222-225.

Livesey CT, Fleming FL (1984) Nutritional influences on laminitis, sole ulcer and bruised sole in Friesian cows. Vet. Rec. 114: 510-512.

Livesey CT, Harrington T, Johnston AM, May SA, Metcalf JA (1998a) The effect of diet and housing on the development of sole haemorrhages and heel erosions in Holstein heifers. Anim. Sc. 67: 9-16.

Livesey CT, Johnston AM, Marsh C, May SA, Metcalf JA (1998b) Hoof horn growth in Holstein cattle from first calving until six weeks after second calving. 10th Int. Symp. Disorders of the Ruminant Digit pp 51-52.

Livesey CT, Laven RA, Marsh C, Johnston AM (2000a) The effects of methionine supplementation and rearing heifers in deep litter straw yards or concrete yards on foot conformation, hoof growth and wear and the development of foot lesions in Holstein cows; evidence of abnormal heel development in heifers reared on straw. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 289-291.

Logue DN, Kempson SA, Leach KA, Offer JE, McGovern RE (1998a) Pathology of the white line. 10th Int. Symp. Disorders of the Ruminant Digit pp 142-145.

Logue DN, Leach KA, Brocklehurst S, Offer JE (2000) Effect of diet on lesion development from birth up to the end of first lactation. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 327-329.

Logue DN, McNulty D, Nolan AM (1998b) Lameness in the dairy cow: pain and welfare. Vet. J. 156: 5-6 (edit.).

Logue DN, Offer JE, Hyslop JJ (1994) Relationship of diet, hoof type and locomotion score with lesions of the sole and white line in dairy cattle. Anim. Prod. 59: 173-181.

Logue DN, Offer JE, Mc Govern RE (1998c) The housing effects of first calving Holstein-Friesian heifers separately or with the adult herd on claw conformation and lesion development. 10th Int. Symp. Disorders of the Ruminant Digit pp 60-62.

Maclean CW (1971) The long term effects of laminitis in dairy cows. Vet. Rec. 89: 34.

Manson FJ, Leaver JD (1988a) The influence of concentrate amount on locomotion and clinical lameness in dairy cattle. Anim. Prod. 47: 185-190.

Manson FJ, Leaver JD (1988b) The influence of dietary protein intake and of hoof trimming on lameness in dairy cattle. Anim. Prod. 47: 191-199.

Manson FJ, Leaver JD (1989) The effect of concentrate: silage ratio and of hoof trimming on lameness in dairy cattle. Anim. Prod. 49: 15-22.

Manson F J, Merritt J B (1990) Feeding factors associated with lameness: a preliminary report from an epidemiological study. Proceedings of 6th International Symposium of Diseases of the Ruminant Digit. pp96-101

Martig J, Leuenberger WP, Dozzi M (1980) Quality and alterations of bovine claws as a function of different variables. Proc. III Int. Symp. on Disorders of the Ruminant Digit. pp 40-55.

Martin SW, Meek AH, Willeberg P (1987) Veterinary epidemiology principles and methods. Iowa State University Press/Ames. Edition 1.

Meyer K, Galbraith H (1998) Claw dimensions and rates of hoof growth and wear in Holstein dairy cows before and after turnout to pasture from concrete cubicles and straw yards. 10th Int. Symp. Disorders of the Ruminant Digit pp 49-50.

Meyer K, Haziengg DG, Galbraith H, Lomax MA (1997) Characterisation of keratin proteins in bovine claw horn taken from the sole. Rep. Scottish Lameness Group Meeting, Hannah Res. Inst. P7.

Midla LT (1997) Efficacy of supplemental biotin for prevention of lesions associated with subclinical laminitis (pododermatitis aseptica diffusa) in first lactation Holsteins. Dissertation, Ohio State University.

Midla LT, Hoblet KH, Weiss WP, Moeschberger M (1998) Supplemental dietary biotin for prevention of lesions associated with aseptic subclinical laminitis (pododermatitis aseptica diffusa) in primiparous cows. AJVR 59: (6) 733-738.

Miller BC, Goodrich RO, Meiske JC (1986a) Effects of dietary additives on B-vitamin production and absorption in steers. J. Anim. Sc. 62: 484-496.

Miller BC, Goodrich RD, Meiske JC (1986b) Effects of grain source and concentrate level on B-vitamin production and absorption in steers. J. Anim. Sc. 62: 473-483.

Money DFL, Laughton GL (1981) Biotin responsive lameness of New Zealand pigs. NZ Vet. J. 29: (3) 33-34.

Mortensen K (1994) Bovine laminitis (diffuse aseptic pododermatitis) clinical and pathological findings. Proc. 8th Int. Symp. Disorders of the Ruminant Digit pp 210-226.

Mosenthin R, Saver WC, Volker L, Frigg M (1990) Synthesis and absorption of biotin in the large intestine of pigs. Liv. Prod. Sc. 25: 95-103.

Mulling C KW (2000) The use of nutritional factors in prevention of claw diseases - biotin as an example for nutrition influences on formation and quality of hoof horn. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 78-80.

Mülling, Ch. K. W., H. H. Bragula, S. Reese, K.-D Budras, and W. Steinberg (1999) How structures in the bovine hoof epidermis are influenced by nutritional factors. Anat. Histol. Embryol. 28:103-108.

Mulling C, Budras KD (1998) Influence of environmental factors on horn quality of the bovine hoof. 10th Int. Symp. Disorders of the Ruminant Digit pp 214-215.

Murphy PA, Hannan J (1986) Effects of slatted flooring on claw shape in intensively housed fattening beef cattle. Proc. Vth Int. Symp. on Disorders of the Ruminant Digit. pp2-7.

Murray RD, Downham DY, Clarkson MJ, Faull WB, Hughes JW, Manson FJ, Merritt JB, Russell WB, Sutherst JE, Ward WR (1996) Epidemiology of lameness in dairy cattle: description and analysis of foot lesions. Vet. Rec. 138: 586-591.

Murray RD, Downham DY, Merritt JR, Russell WB, Manson FJ (1994) Observer variation in field data describing foot shape in dairy cattle. Res. Vet. Sc. 56: 265-269.

Nocek JE, Tomlinson DJ, Johnson AB, Socha MT (2000) Evaluation of claw characteristics in high-producing herds as influenced by the supplementation of complexed trace minerals. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 308-310.

Offer JE, Fisher GEL, Kempson SA, Logue DN (2001) The effect of feeding grass silage in early pregnancy on claw health during first lactation. Vet. J. 161 (2): 186-193.

Offer JE, Fisher GEJ, Kempson SA, Logue DN (2001) The effect of feeding silage in early pregnancy on claw health during first lactation. Vet. J. 161 (2) 186-193.

Offer JE, Logue DN (1998) The effect of lameness in the dairy cow on the fatty acid profile of claw horn lipids. 10th Int. Symp. Disorders of the Ruminant Digit pp 220-221.

Offer JE, Logue DN, Leach KA (2000) Changes in claw conformation, growth and wear in youngstock and first lactation cattle. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 330-332.

Offer JE, Mc Nulty D, Logue DN (1998) Modelling lesion development in a cohort of Holstein-Friesian cows (lactations 1to 4). 10th Int. Symp. Disorders of the Ruminant Digit pp 159-160.

Offer JE, Offer NW, Logue DN (2000) Effects of dietary fish oil supplementation on the hoof lipid fatty acid profiles of dairy cattle (poster). XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 322-324.

Offer JE, McNulty D Logue DN (2000) Observations of lameness, hoof conformation and development of lesions in dairy cattle over four lactations. Vet. Rec. 147 (4):105-109.

Ossent P, Lischer CJ (1994) Theories on the pathogenesis of bovine laminitis. Proc. 8th Int. Sym. Disorders of the ruminant digit, pp207-209.

Ossent P, Lischer CJ, Raber M, Geyer H (2000) The significance of the suspensory mechanism of the third phalanx in the pathogenesis of sole ulcers in cattle. Part II: Microscopic findings. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 226-229.

Ossent P, Peterse DJ, Schamhardt HC (1987) Distribution of load between the lateral and medial hoof of the bovine hind limb. J. Vet. Med. 34, 296-300.

Parizi MA, Hosseini M (1998) Survey of lameness in six dairy farms of Shiraz area. 10th Int. Symp. Disorders of the Ruminant Digit pp 75-77.

Peeler EJ, Otte MJ, Esslemont RJ (1994) Inter-relationships of periparturient diseases in dairy cows. Vet. Rec. 134: 129-132.

Penny RHC, Cameron RDA, Johnson S, Kenyon PJ, Smith HA, Bell AWP, Cole JPL, Taylor J (1980) Foot rot of pigs: the influence of biotin supplementation an foot lesions in sows. Vet. Rec. 107: 350-351.

Peterse DJ (1985) Laminitis and interdigital dermatitis and heel horn erosion. Vet. Clin. North Am. Food Anim. Prac. 1: (1) 83-91.

Phillips CJC, Chiy PC, Bucktrout MJ, Collins SM, Gasson CJ, Jenkins AC, Paranhos Da Costa MJR (2000) Frictional properties of cattle hooves and their conformation after trimming. Vet. Rec. 146, 607-609.

Phillips CJC, Coe R, Colgan M, Duffus C, Ingoldby L, Pond M, Postlethwaite S (1998) Effect of hoof characteristics on the propensity to slip in cattle. Vet Rec 141:242-245.

Phillips CJC, Patterson SJ, Dowi IAP, Whitaker CJ (1996) Volume assessment of the bovine hoof. Res. Vet. Sc. 61: 125-128.

Politiek RD, Distl O, Fjeldaas T, Heeres J, McDaniel BT, Nielsen E, Peterse DJ, Revrink A, Strandberg P (1986) Importance of claw quality in cattle: review and recommendations to achieve genetic improvement. Report of the EAAP working group on 'claw quality in cattle'. Liv. Prod. Sc. 15: 133-152.

Prentice DE, Neal PA (1972) Some observations on the incidence of lameness in dairy cattle in west Cheshire. Vet. Rec. 91: (1) 1-7.

Roberts CJ, Baggott DG (1982) Biotin - experiments with dairy cattle. Roche Vit. Symp. London pp 13-20.

Rodriguez-Lainz A, Melendez-Retamol P, Hird DW, Read DH, Walker RL (1999) Farm- and host-level risk factors for papillomatous digital dermatitis in Chilean dairy cattle. Prev. Vet. Med. 42: 87-97.

Rowlands GJ, Russell AM, Williams LA (1983) Effects of season, herd size, management system and veterinary practice on the lameness incidence in dairy cattle. Vet. Rec. 113: 441-445.

Russell AM (1986) Influence of sire on lameness in cows. Proc. British Cattle Vet. Assoc. pp 213-220.

Russell AM, Rowlands GJ, Shaw SR, Weaver AD (1982) Survey of lameness in British dairy cattle. Vet. Rec. 111: 155-160.

Sarasin A (1994) An in vitro model for organotypic epidermal differentiation: effects of biotin. Dissertation, University Zurich.

Scaife JR, Galbraith H, Tahmasbi MA, Mengal MA (1997) Role of biotin in hair growth and protein deposition in sheep and goat hair follicles. F-Hoffmann La Roche doc. Uni. Aberdeen, Dept. Agric.

Scaife JR, Meyer K, Grant E (2000) Comparison of the lipids of the bovine and equine hoof horn (poster). XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 125-127.

Schmid M, Geyer TH (1994) Effect of biotin on the quality of the claw horn in dairy cows. 20th Congress Euro. Assoc. Vet. Anatomists, Zurich 15-19.

Scholtissek J, Barth CA, Hagemeister H (1990) Biotin supply by large bowel bacteria in minipigs: evidence from intracaecal avidin. Brit. J. Nutr. 64: 715-720.

Schulze J, Scherf H (1989) Klinische studie zur therapie mit biotin beim pferd. Tierarztl. Umschau 4, 187-190.

Seymour WM (1998) Role of biotin in ruminant nutrition examined. Feedstuffs 70: (19) 14-18.

Simmins PH, Brooks PH (1983) Supplementary biotin for sows: effect on reproductive characteristics. Vet. Rec. 112: 425-429.

Singh SS, Ward WR, Hughes JW, Lautenbach K, Murray RD (1994a) Behaviour of dairy cows in a straw yard in relation to lameness. Vet. Rec. 135: 251-253.

Singh SS, Ward WR, Lautenbach K, Hughes JW, Murray RD (1993) Behaviour of first lactation and adult dairy cows while housed and at pasture and its relationship with sole lesions. Vet. Rec. 133: 469-474.

Singh SS, Ward WR, Murray RD (1994b) An angiographic evaluation of vascular changes in sole lesions in the hooves of cattle. Vet. Rec. 150: 41-52.

Smilie RH, Hoblet KH, Weiss WP, Schnitkey GL, Moeschberger ML (1999) Subclinical laminitis in dairy cows; use of severity of hoof lesions to rank and evaluate herds. Vet. Rec. 144 (1): 17-21.

Steinberg W, Griggio C, Bohn N, Klunter AM, Schuep W (1996) Influence of graded biotin supplementation on biotin concentrate in blood plasma and milk of dairy cows. April 2, (Hoffmann-La Roche Research report).

Steinberg W, Klunter AM, Schuep W (1994) Excretion of biotin and plasma kinetics in lactating dairy cows after intravenous and intraruminal application. Proc. Soc. Nutr. Physiol. 3 p226.

Steinberg W, Klunter AM, Bohn N, Griggio C, Schuep W (1995) Biotin balance studies in dairy cows with and without biotin supplementation. June 19, Hoffman-La Roche, Research report.

Sugg JL, Brown AH, Perkins JL, Phillips JM, Kellogg DW, Johnson ZB (1996) Performance traits, hoof mineral composition, and hoof characteristics of bulls in a 112-day postweaning feedlot performance test. AJVR 57: (3).

Thomason JJ, Biewener AA, Bertram JEA (1992) Surface strain on the equine hoof wall *in vivo*: implications for the material design and functional morphology of the wall. J. Exp. Biol. 166, 145-168.

Toussaint Raven E (1973) Lameness in cattle and foot care. Netherlands J. Sc. 5, 105-111.

Toussaint Raven E (1985) Cattle foot-care and claw trimming. Farming Press Ltd. Ed. 1, pp1-21.

Tranter WP, Morris RS (1992) Hoof growth and wear in pasture-fed dairy cattle. NZ Vet. J. 40: 89-96.

Tranter WP, Morris RS, Williamson NB (1991) A longitudinal study of the hooves of non-lame cows. NZ Vet. J. 39: 53-57.

Vermunt J J (1990) Lesions and structural characteristics of the claws of dairy heifers in two management systems. Msc Thesis, University of Saskatchewan.

Vermunt JJ (1992) 'Subclinical' laminitis in dairy cattle. NZ Vet. J. 40: 133-138.

Vermunt JJ (1998) Axial wall crack. 10th Int. Symp. Disorders of the Ruminant Digit pp 141.

Vermunt JJ (2000) Risk factors for laminitis - An overview. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 34-45.

Vermunt JJ, Greenough PR (1990) Observations on management and nutrition in a Holstein dairy herd affected by subclinical laminitis. Proc. VIth Int. Symp. on Diseases of the Ruminant Digit. pp 22- 35.

Vermunt JJ, Greenough PR (1994) Predisposing factors of laminitis in cattle. Brit. Vet. J. 150: 151-164.

Vermunt JJ, Greenough PR (1995a) Lesions associated with subclinical laminitis of the claws of dairy calves in two management systems. Brit. Vet. J. 151: 391-399.

Vermunt JJ, Greenough PR (1995b) Structural characteristics of the bovine claw: horn growth and wear, horn hardness and claw conformation. Brit. Vet. J. 151: 157-180.

Vermunt JJ, Greenough PR (1996a) Sole haemorrhages in dairy heifers managed under different underfoot and environmental conditions. Brit. Vet. J. 152: 57-73.

Vermunt JJ, Greenough PR (1996b) Claw conformation of dairy heifers in two management systems. Brit. Vet. J. 152: 321-331.

Vincent JFV (1992) Biomechanics -Materials A Practical Approach. IRL Press: Oxford, UK, pp 57-74.

Voigt J, Hagemeister H, Kuhla S, Schoenhusen U, Weber GM (2000) The effect of biotin supplementation on claw health in dairy cattle kept under field conditions. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 292-294.

Warzecha C (1993) The ruminant hoof: morphological and histochemical findings in cattle, sheep and goat. Dissertation, Uni. Zurich.

Weaver D (1997) Lameness and foot trimming – what farmers do about it. Dairy Farmer January 7th pp52.

Weaver D (1998) Analysis of some epidemiology factors in lameness on 55 Somerset (England) dairy herds. 10th Int. Symp. Disorders of the Ruminant Digit pp 65-69.

Weaver D (1990) Long-term observations on dairy herd lameness. Proceedings of the 6th International Symposium on Disorders of the Ruminant digit. Liverpool, UK, pp59-61.

Webb NG, Penny RHC, Johnston AM (1984) Effect of a dietary supplement of biotin on pig hoof horn strength and hardness. Vet. Rec. 114: 185-189.

Webster AJF (2000) Effects of wet v. Dry feeding and housing type on the pathogenesis of claw horn disruption in first-lactation dairy cattle. XI Int. Symp. Disorders of the Ruminant Digit and III Int. Conf. on Bovine lameness pp 340-345.

Weiss WP, Zimmerly CA (2001) Effects of biotin on metabolism and milk yield of dairy cows. Ohio State University, OARDC, Columbus, Ohio.

Whay HR, Waterman AE, Webster AJF (1997) Associations between locomotion, claw lesions and nociceptive threshold in dairy heifers during the peri-partum period. Vet. J. 154: 155-161.

Whitehead CC (1988) Biotin in Animal Nutrition. Hoffmann-La Roche Publication ISBN 3-906507-02-5.

Zenker W (1991) Hufhornveranderugen bei Lippizanerpferden und ein Behandlungsversuch mit Biotin. DVM Thesis, University of Zurich.

Zenker W, Josseck H, Geyer H (1995) Histological and physical assessment of poor hoof horn quality in Lippizzaner horses and a therapeutic trial with biotin and a placebo. Eq. Vet. J. 27: (3) 183-191.

Zinn RA, Owens FN, Stuart RL, Dunbar JR, Norman BB (1987) B-vitamin supplementation of diets for feed-lot calves. J. Anim. Sc. 65: 267-277.

APPENDICES

APPENDIX I

- Recording form for parlour use of biotin.
- Recording form for calibration of the parlour dispensing points.
- Recording form for dry cow and heifer feed use and movement from farm to farm.
- Recording form for lameness diagnosis

APPENDIX II

- Ingredients of the dry cow and heifer feed ration from the 18 month trial.
- Ingredients of the dry cow and heifer feed ration from the 6 month trial.

APPENDIX III

- Meeting agenda.
- Meeting minutes.

APPENDIX IV

- Table of secondary lesions.
- Table of 'other' lesions.
- Table of repeat visits to a secondary lesion.

APPENDIX V

- Horn cutters for biomechanical tests A and B.
- General linear model analysis of individual sample periods 0-5.

APPENDIX VI

- Blowey RW, Ossent P, Watson CL, Hedges VJ, Green LE, Packington AJ (2000) Possible distinction between sole ulcers and heel ulcers as a cause of bovine lameness. Vet. Rec. 147, 110-112.

APPENDIX I

Parlour Solution (2%) Record in Litres

Form ID S5001
Farm No 5

Date	Temp	Previous Reading	Current Reading	Animal Numbers	Expect Use	Actual Use	Vari (%)	Biotin Amount	Added Code/ref	New Reading	Recali Date	bration Ref
11/8/97	-	-	26	-	-	-	-	65	B24	26	11/8/97	C5001/2
27/8/97	-	26	19	22	3.3	7	52.8	-	-	19	24/9/97	-
2/9/97	-	19	10	22/25	7.6	9	15.6	-	-	10	24/9/97	-
9/9/97	-	10	9	27	9.5	1	(fault)	22	B36	31	24/9/97	-
16/9/97	-	31	16	27	9.5	15	36.7	22	B41	38	24/9/97	(delay)
22/9/97	-	38	28.5	27/30	8.7	9.5	8.4	22	B41	52.5	22/9/97	C5003
29/9/97	-	52.5	39	30	10.5	13.5	22	-	-	39	20/10/97	-
7/10/97	-	39	25	30/33	13	14	7	22	B49	49	20/10/97	-
13/10/97	-	49	38	33	12.05	11	9.5	23	B55	61	20/10/97	-
20/10/97	-	61	49	33/36/31	12.3	12	2.5	-	-	49	20/10/97	C5004
27/10/97	10.9	49	37	31/33	11.45	12	4.5	-	-	37	24/11/97	-
3/11/97	15	37	22	33/35	15.78	15	5	22	B67	45	24/11/97	-
10/11/97	15	45	33	36/37	12.85	12	6	-	-	33	24/11/97	-
17/11/97	15	33	19	37	13	14	7.5	22	B79	43	24/11/97	-
24/11/97	17	43	28	35	11.95	15	20.3	22	B81	49	24/11/97	C5005
2/12/97	18.5	49	31	34	13.6	18	24.4	22	B85	52	17/12/97	-
9/12/97	15	52	38	34	11.9	14	15	22	B91	58	17/12/97	-
17/12/97	15	58	42	34	13.4	16	16.25	22	B93	62	17/12/97	C5006
23/12/97	16	62	52	34	10.1	10	0.9	22	B102	70	19/1/98	-
31/12/97	17	70	54	35	14.05	16	12.2	0	0	54	19/1/98	-
6/1/98	16	54	41	36	10.8	13	16.9	0	0	41	19/1/98	-
12/1/98	18	41	28	36	10.8	13	16.9	0	0	28	19/1/98	-
19/1/98	17	28	14	36	12.6	14	10	22	B111	33.5	19/1/98	C5007
28/1/98	16	33.5	15	35	15.7	18.5	15.1	44	B119	59	10/2/98	-

Calibration of the Parlour System Form ID C1004

Date 16/9/97 Farm No 1

Measurements taken in number order				Measurements taken in alternate order			
LEFT (ml)		RIGHT (ml)		LEFT (ml)		RIGHT (ml)	
1	25	1	25	1	24	1	25
2	24	2	26	2	24	2	26
3	25	3	25	3	25	3	26
4	24	4	26	4	25	4	26
5	24	5	27	5	25	5	26.
6	27	6	27	6	26	6	27
7	26.	7	25.	7	27.	7	26
8		8		8		8	
9		9		9		9	
10		10		10		10	
11		11		11		11	
12	AV. 25	12	AV. 25.8	12	AV. 25.14	12	AV. 26

Recalibration Date 14/10/97. Monthly Check Yes/~~No~~

Prior Sample B Ref 1342

E Ref

E Date

Dry Cow Roll Record in Kg

Form ID F2001 .....
Farm No 2.....

Date	Biotin Suppl	Animal Numbers	Current Amount (KG)	Expect Use	Actual Use	Diff (Kg)	Feed Delivery		Delivery Date	New Amount	New roll	Samples
							Amount	Batch no			Date	Ref
11/7		-	-	-	-	-	1T (of each)	1281/82	11/7/97	2T	11/9/97	D2001
14/8	Y	8	800	-	-	-	-	1282	-	-	-	-
14/8	N	12	737					1281	-	-	-	-
26/8	Y	7	750	49	50	1	-	1282	-	700	-	-
26/8	N	9	675	54	62	8		1281	-	675	-	-
8/9	Y	8	610	52	65	13		1282	-	610	-	-
8/9	N	7	637.5	45.5	37.5	-8		1281	-	637.5	-	-
12/9	Y	8	585	16	25	9		1282	11/9/97	1585	12/9/97	D2002
12/9	N	7	620	14	17.5	3.5		1281	11/9/97	1620	12/9/97	D2002
22/9	N	6	1580	30	40	10		1281	-	1580	-	-
22/9	Y	6	1540	30	45	15		1282	-	1540	-	-
6/10	N	4	1560	28	30	2		1281	-	1550	-	-
6/10	Y	6	1490	42	50	8		1282	-	1490	-	-
6/10	Y	moved	50	to F3	-	-	-	1282	-	1440	-	-
6/10	N	moved	100	to F3	-	-	-	1281	-	1450	-	-
17/10	Y	moved	75	to F4	-	-	-	1282	-	1365	-	-
17/10	N	moved	50	to F4	-	-	-	1281	-	1400	-	-
20/10	N	4	50	28	30	2		1281	-	1360	-	-
20/10	Y	6	260	42	50	8		1282	-	1315	-	-
22/10	Y	moved	100	to F4+5	-	-	-	1282	-	1215	-	-
24/10	Y	moved	50	to F4	-	-		1282	-	1165	-	-
27/10	Y	moved	25	to F1	-	-		1282	-	1145	-	-

BIOTIN TRIAL - VETERINARY FORM FORM ID.....

N.B. FILL IN ONE FORM PER AFFECTED **DIGIT** No. Photos.....

1. Farm ID _____

2. Date (dd/mm/yy) _____/_____/ ____

3. Cow ID _____ freeze brand/ear tag (delete as appropriate)

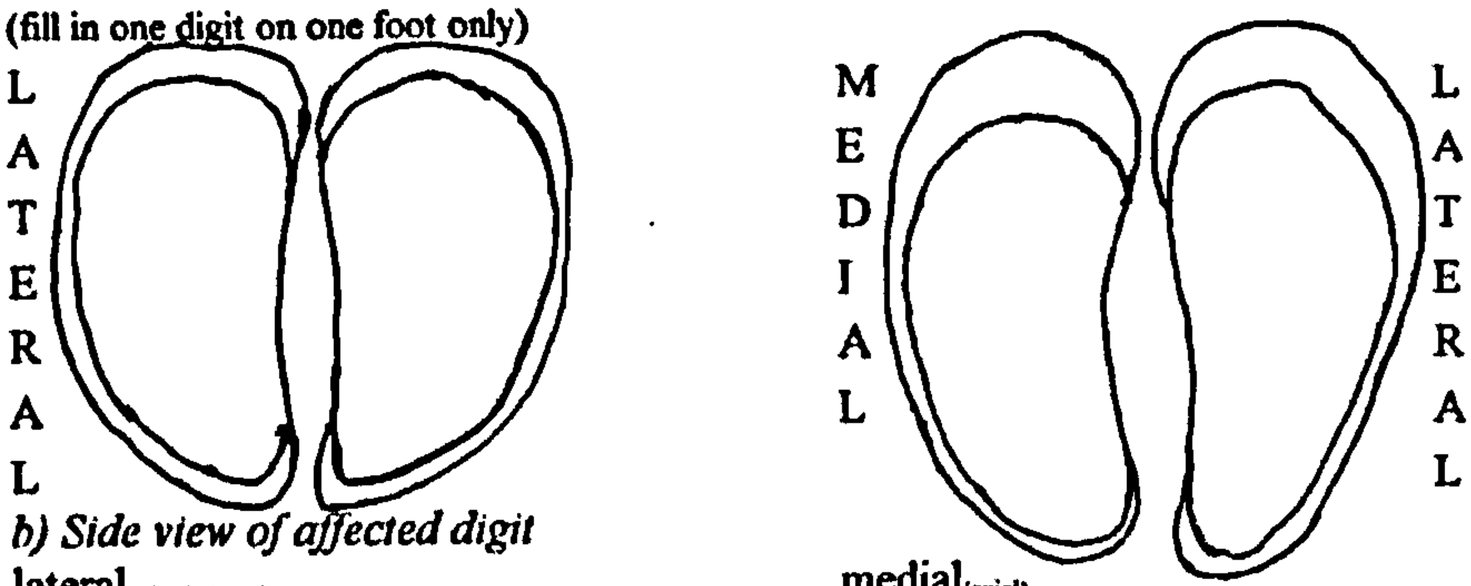
4. Lame Foot (circle one only) **LF** **RF**

LH **RH**

5. Indicate affected area ⇩⇩ ⇩⇩
(mark clearly with pen) ⇩⇩ ⇩⇩

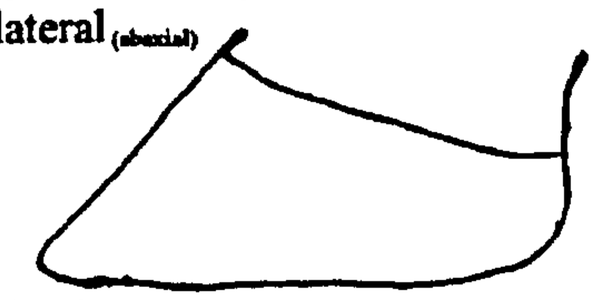
a) Volar surface LEFT FOOT RIGHT FOOT

(fill in one digit on one foot only)



b) Side view of affected digit

lateral(abaxial)



6. Diagnosis (circle one or more)

1. solar ulcer
2. white line
3. digital dermatitis
4. foul
5. slurry heel
6. foreign body
7. under run sole
8. under run wall
9. necrotic heel track
10. vertical fissure
11. axial groove fissure
12. horizontal wall fissure
13. solar haemhorrage
14. interdigital growth
15. Other _____(please specify)

7. Treatment (circle one or more)

1. pare
2. spray
3. inject
4. block
5. Anti-Inflammatory
6. A/Biotic _____ (please specify)
7. Other _____

8. Vet ID (circle one only)
RB AC PC CH EJP
GS CW Other _____(please specify)

APPENDIX II

Biotin trial feed formula (biotin supplemented)

BOCM Pauls Ltd, Portbury Mill.

PC ROLL + BIOTIN

NAME	INCLUSION (%)
Barley	22.470
Wheatfeed	11.100
Sugar rich dairy: biscuit meal	10.000
High quality rice bran	15.000
Rape ext (45 µm) hearmeal	13.300
Copra	2.600
Sunflower	16.000
Molasses: CMS 80:20 blend	5.000
BOCM PAULS mixer fat: blend 25	0.400
BOCM PAULS spray fat: blend 25	1.000
Calcined magnesite	1.300
DI calcium phosphate	0.750
Salt	0.680
ROVIMIX H-2 (biotin)	0.200
BOCM PAULS cattle premix (maize)	0.100
Sorbic acid	0.100
	100.00

Biotin trial feed formula (unsupplemented)

BOCM Pauls Ltd, Portbury Mill.

PC ROLL ROCHE

NAME	INCLUSION (%)
Barley	22.470
Wheatfeed	11.100
Sugar rich dairy: biscuit meal	10.000
High quality rice bran	15.000
Rape ext (45 μm) hearmeal	13.300
Copra	2.600
Sunflower	16.000
Molasses: CMS 80:20 blend	5.000
BOCM PAULS mixer fat: blend 25	0.400
BOCM PAULS spray fat: blend 25	1.000
Calcined magnesite	1.300
DI calcium phosphate	0.750
Salt	0.680
BOCM PAULS cattle premix (maize)	0.100
Sorbic acid	0.100
	100.00

6 month trial (unsupplemented ration)

: 12:29 06/04/00 (FM) 0000 Ruminant Dev (1) ALL DATA
Single-Mix 0001 :
: Plant=88
19-October-1998/47.3 :

Formula basic data

Code : 9000 Name : CHERRY ORCHARD PLAIN (CONTROL)

Sell price: 0.0 Batch [Kg]: 750.0 Group code:
Cost : 33.029 Created : 06/04/00 Version :
Margin : -33.029 Updated : 06/04/00 FM origin :
1
Tonnes : 0.0 User name : VM key :
1

External reference:
Script file name :

Analysis

[VOLUME]		: 100.0	FIBRE	%	: 5.98125	CALC	% : 1.026425
DRY MAT	%	: 87.5	ASH	%	: 7.995275	PHOST	% : 0.5923
PROTEIN	%	: 16.09	STARCH	%	: 28.29045	SALT	% : 1.34615
OIL (EE)	%	: 2.58775	SUGAR	%	: 8.47525	MAG	% : 0.43955
OIL (B)	%	: 3.078	ME(R)	MJ/Kg	: 11.0258		

	Raw material	Available	%	[Kg]	Tonnes	
1	BARLEY 10%	[X]	10.0	75.0	0.0	
19	WHEAT MIDDLINGS	[X]	20.0	150.0	0.0	
22	WHEAT 10% 2	[X]	30.85	231.375	0.0	
76	MOLASSES	[X]	2.5	18.75	0.0	
77	SUG. BEET PULP, MOL.	[X]	15.0	112.5	0.0	
100	SOYA-HIPRO	[X]	16.0	120.0	0.0	
106	DICALCIUM PHOSPHATE 40	[X]	1.0	7.5	0.0	
110	LIMESTONE	[X]	1.5	11.25	0.0	
116	SALT	[X]	1.2	9.0	0.0	
155	FAT BLEND	[X]	1.0	7.5	0.0	
235	CALCINED MAGNESITE	[X]	0.5	3.75	0.0	
4800	MYCO-CURB	[X]	0.1	0.75	0.0	
4802	SORBIC ACID	[X]	0.1	0.75	0.0	
5111	CUSTOM MIX	[X]	0.25	1.875	0.0	
			100.0	750.0		

6 month trial (Biotin supplemented ration)

: 12:29 06/04/00 (FM) 0000 Ruminant Dev (1) ALL DATA
Single-Mix 0002 :
: Plant=88
19-October-1998/47.3 :

Formula basic data

Code : 9001 Name : CHERRY ORCHARD EXTRA (Biotin TREATMENT)

Sell price: 0.0 Batch [Kg]: 750.0 Group code:
Cost : 33.029 Created : 06/04/00 Version :
Margin : -33.029 Updated : 06/04/00 FM origin : 1
Tonnes : 0.0 User name : VM key : 1

External reference:
Script file name :

Notes

1: The approximate targets for both are as follows: Protein 16%, Ca 0.8-1.2%,
2: P 0.55-0.65%, Mg 0.4-0.55%, Salt 1.0-1.5%. Please add dairy min/vit sup.
3: Custom mix=low inclusion min/vit supplement but you can use a higher
4: inclusion product with major minerals balanced if you wish.

Analysis

[VOLUME]	:	100.0	FIBRE %	:	5.9785	CALC %	: 1.02637
DRY MAT %	:	87.5143	ASH %	:	7.99357	PHOST %	: 0.59197
PROTEIN %	:	16.079	STARCH %	:	28.22698	SALT %	: 1.34604
OIL (EE) %	:	2.5861	SUGAR %	:	8.4725	MAG %	: 0.43944
OIL (B) %	:	3.0758	ME(R) MJ/Kg	:	11.01282		

Tonnes		Raw material	Available	%	[Kg]
	1	BARLEY 10%	[X]	10.0	75.0
0.0					
	19	WHEAT MIDDLINGS	[X]	20.0	150.0
0.0					
	22	WHEAT 10% 2	[X]	30.74	
230.55	0.0				
	76	MOLASSES	[X]	2.5	
18.75	0.0				
	77	SUG. BEET PULP, MOL.	[X]	15.0	112.5
0.0					
	100	SOYA-HIPRO	[X]	16.0	120.0
0.0					
	106	DICALCIUM PHOSPHATE 40	[X]	1.0	7.5
0.0					
	110	LIMESTONE	[X]	1.5	
11.25	0.0				
	116	SALT	[X]	1.2	9.0
0.0					
	155	FAT BLEND	[X]	1.0	7.5
0.0					
	235	CALCINED MAGNESITE	[X]	0.5	
3.75	0.0				
	4800	MYCO-CURB	[X]	0.1	
0.75	0.0				
	4801	ROVIMIX H-2 (BIOTIN)	[X]	0.11	
0.825	0.0				
	4802	SORBIC ACID	[X]	0.1	
0.75	0.0				
	5111	CUSTOM MIX	[X]	0.25	
1.875	0.0				
				100.0	750.0
0.0					

APPENDIX III

Agenda

Thursday 2nd July 1998

Hatherley Manor Hotel

Roche/Wood Veterinary Group Trial Liaison Meeting

5.30	Introduction	Roger Blowey
	Review of previous action points	All
	Trial Progress Report	Ginny Hedges
	Documentation/Photographic Issues	Ginny Hedges
	Forthcoming trial procedures	Ginny Hedges
	Locomotion assessment	R Blowey
	Necrotic heel discussion	R Blowey
	General discussion	All

Farmers Biotin Meeting

7.30	Introduction	Adrian Packington
	Progress report and Presentation of paper to be given at 10th International Lameness Symposium	Ginny Hedges
	The Dimensions of Statistics'	Laura Green
	Forthcoming trial procedures	Ginny Hedges
	Questions and Answers	All
	The process of horn development and the formation of sole ulcers	R Blowey

ROCHE BIOTIN TRIAL

VME Field Trial Review Meeting

Date: 2 July 1998

Venue: Hatherley Manor Hotel, Gloucester

Present: Adrian Packington, Laura Green, Ginny Hedges
Roger Blowey, Chris Watson, Graham Stephens, Paul Cunliffe

1 Introduction

This was the third meeting since the commencement of the trial, to review all progress to date, issues raised within the previous meeting and further issues that required discussion.

2 Review of previous action points

Lameness report forms and photos

Photographic presentation of the foot and lesion have improved in most cases.

- Foot >50% of the photo
- Definite marker pen labeling put alongside the foot

Missing report forms and slides have been located.

Necrotic Heel Track:- uniformity of diagnosis.

Lateral/medial, abaxial/axial wall identified.

GENERAL DISCUSSION

3 When is a cow lame?

3.1 An initial discussion was made to distinguish between a stiff cow and a lame cow, taking into consideration track surface.

3.2 Consultation of the herdsman, by all vets, distinguished those cows which were commonly stiff.

3.3 Those identified as lame by the farmer are observed walking into the crush, examined and in the event of no lesion let out of the crush for further walking observation and re-examined where necessary (GS, CW). The foot is examined even in a higher limb lameness case.

4 Diagnosis of Sole Haemhorrage

4.1 In some lameness reports no definite cause has been found, however, there appears a slight haemhorrage; but, is this the cause of the lameness?

4.2 This lesion is not reported in the event of another lesion being present (RB, GS, PC).

5 Lameness Assessment

Different techniques of Lameness walkpast

- Picking out lame cows already identified by herdsman(observed PM) (GS,PC)
- Assessment after those considered lame by the herdsman and have already been removed (observed AM) (RB)
- Whole herd check, notes cows that display lameness and herdsman later confirms (CW)

5 Lesion Assessment

5.1 Attempts are made to keep to one lesion reporting per lame cow where possible
- Slurry heel and sole haemhorrage are lesions which tend to be difficult to report or diagnose

5.2 Suggestions were made to reduce the lesions recorded to those of most significance for the purpose of analysis

Action: V Hedges

5.3 Should under-run-sole be added to the form if the original lesion is a white line?
Currently:
- White line only written (RB,CW,PC)
- White line written only when small area of URS and both reported where a large area of URS is observed (GS)

Standardization of diagnosis of white line lesion with an accompanying under-run-sole of different severity's.
- 1/2 sole effected URS reported
- Approx. 1 inch diameter or less effected URS not reported

Action: All vets

5.4 Other causes of lameness
- This category accounts for 14% of the total observed lesions
- Included are upper-limb lesions, healing, e.g. granuloma and lesions unlisted on the reporting form
- Reorganization of the 'other' category in the current database and forms so that irrelevant data to this trial is not included

Action: V Hedges

5.5 Repeat visits

- What is a suitable time interval to report a lesion as new?
- Cows are sometimes resubmitted for examination before the lesion has healed (CW)
- Approx. 2 weeks can be allowed or the cow would be examined if the lesion is getting worse and not better

Action: All vets

6 Foot-trimming

- Warren's dry-off foot-trim was arranged only to occur last year
- All foot-trimming that is carried out that is not a lameness reporting should be recorded on the visiting/treatment chit incorporating the cow ID and the trimmed claw (LF,RF,LH,RH)

Action: All vets

- Hartpury and King have records of foot-trimming on pink chits to be recorded

Action: V Hedges

7 Axial Groove Fissure

Discussion on the pathogenesis, aetiology of an axial groove fissure. This lesion appears to be a split in the hoof wall, leading to pinching of the corium, rather than an infection and granulation at the axial coronary band.

MERRY CHRISTMAS!

APPENDIX IV

Incidence of lameness lesions reported as secondary lesions to the main lameness lesion on all farms combined by supplementation.

Factor	Biotin Supplement	Unsupplemented	P	Total
Sole Ulcer	0.70	2.35	0.04*	1.52
White line	0	0.18	0.50[a]	0.09
Digital Dermatitis	0.88	0.36	0.45[a]	0.62
Interdigital necrobacillosis	0.35	0	0.50[a]	0.18
Foreign body	0	0.72	0.06[a]	0.36
Horizontal wall fissure	0	0.18	0.50[a]	0.09
Interdigital growth	0.70	1.09	0.54[a]	0.89
Heel ulcer	0	0	0	0
Under run wall	0.35	0.18	1.00	0.27
Under run sole	5.98	6.16	0.94	6.07
Slurry heel	1.58	0.72	0.27	1.16
Sole haemorrhage	1.41	1.63	0.98	1.52
Vertical wall fissure	0	0	0	0
Other	2.82	4.17	0.30	3.48

*P Significant, P = Yates corrected, [a] 2-tailed fisher exact.

Lesions diagnosed in the 'other' category with all farm data combined and include primary and secondary 'other' lesions and those that arise at repeat visits.

Diagnosis	Biotin Supplemented	Unsupplemented	P	Total Incidence
Swollen fetlock	0.52	0.18	0.62[a]	0.36
Sole ledge/Overgrowth	3.87	1.99	0.08	2.95
Granulation tissue	1.23	1.27	0.81	1.25
No diagnosis	1.06	2.53	0.11	1.78
Swollen hock	0.70	0.36	0.69[a]	0.53
Coronary band trauma	0.18	0.90	0.12	0.53
Septic arthritis	0	0.36	0.25[a]	0.18
Block trauma	0.35	0.54	0.68[a]	0.45
Hip injury	0.18	0	1.00[a]	0.09
Abcess	0.52	0.54	1.00[a]	0.53
Hock damage	0	0.36	0.25[a]	0.18
Thin soles	0	0.18	0.50[a]	0.09
Nerve damage	0.18	0.36	0.62[a]	0.27
Sole penetration	0	0.72	0.06[a]	0.36
Foot trauma	0.52	0.18	0.62[a]	0.36
Pus and joint sepsis	0.18	0	1.00[a]	0.09
Under run heel	0	0.18	0.50[a]	0.09
Joint injury	0.18	0	1.00[a]	0.09
Toe pus	0.35	0.36	1.00[a]	0.36
False toe	0	0.18	0.50[a]	0.09
Claw polyp	0	0.18	0.50[a]	0.09
Fetlock damage	0.18	0	1.00[a]	0.09
Laminitis	0.53	0	0.25[a]	0.27
Leg oedema	0.35	0.18	1.00[a]	0.27
Swollen knee	0	0.36	0.25[a]	0.18
Pedal bone necrosis	0	0.54	0.12[a]	0.27
Arthritic changes	0	0.18	0.50[a]	0.09
Septic foot	0.18	0.18	1.00[a]	0.18
Soft sole	0.35	0.18	1.00[a]	0.27
Heel bruising/trauma	0	0.18	0.50[a]	0.09

*P significant, P = Yates corrected, [a] 2-tailed fisher exact, chi square.

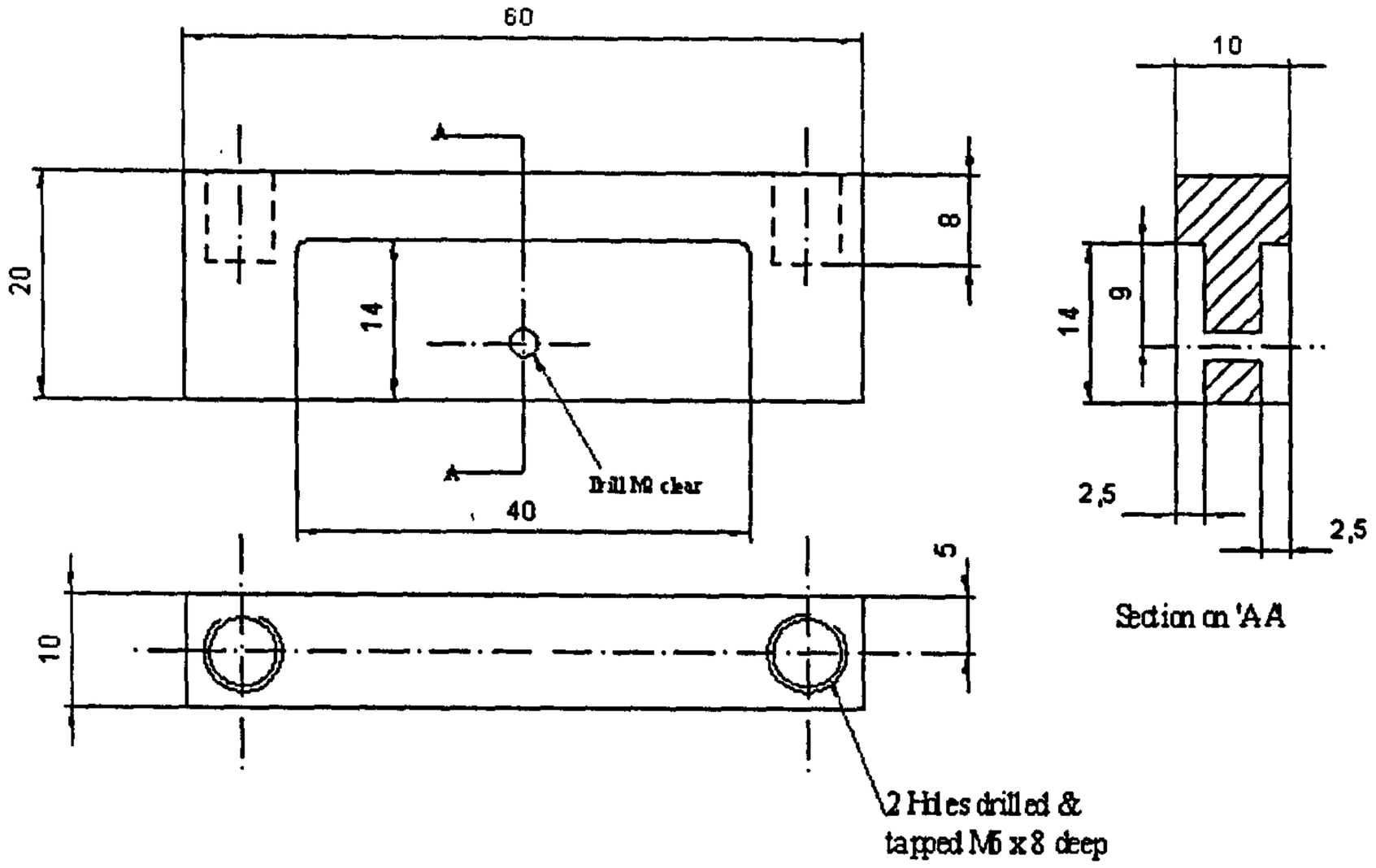
Incidence of repeat lameness lesions reported as secondary lesions to the main lameness lesion on all farms combined by supplementation.

Factor	Biotin Supplement	Unsupplemented	P	Total
Sole Ulcer	0	0	0	0
White line	0	0	0	0
Digital Dermatitis	0	0.18	0.50[a]	0.09
Interdigital necrobacillosis	0.35	0	0.50[a]	0.18
Foreign body	0	0.18	0.50[a]	0.09
Horizontal wall fissure	0	0	0	0
Interdigital growth	0	0.18	0.50[a]	0.09
Heel ulcer	0.18	0.18	1.00[a]	0.18
Under run wall	0	0.72	0.06[a]	0.36
Under run sole	0.35	0.90	0.28[a]	0.62
Slurry heel	0.18	0	1.00[a]	0.09
Sole haemorrhage	0.35	0.18	1.00[a]	0.27
Vertical wall fissure	0	0	0	0
Other	0.53	0.72	0.72[a]	0.62

*P Significant, P = Yates corrected, [a] 2-tailed fisher exact.

APPENDIX V

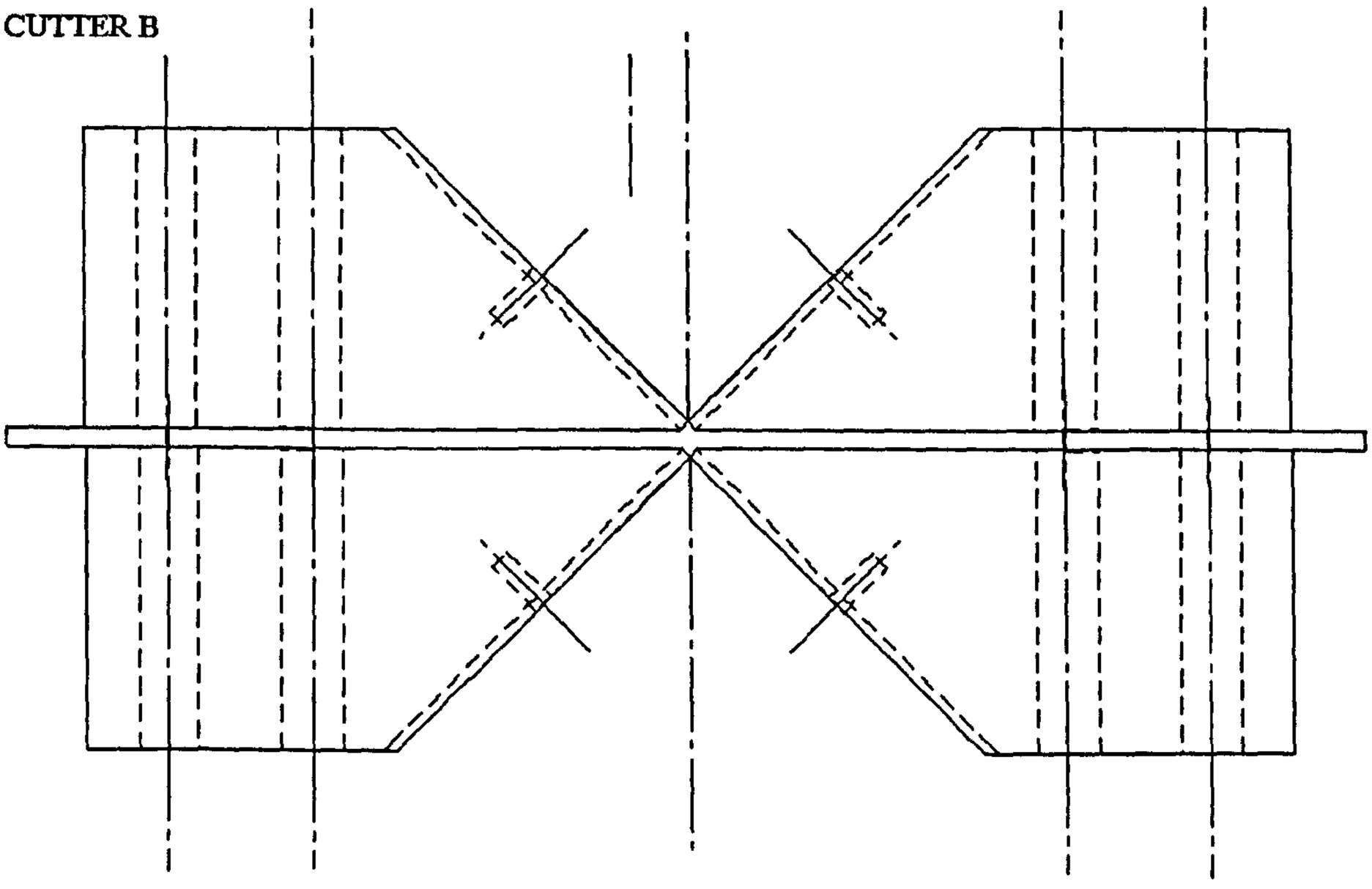
CUTTER A



Razor blade holder

2 Off mild steel

CUTTER B



Analysis of variance (general linear model) for peak tensile strength (mega-pascals) of the white line for sample day 0

Variable	D F	Adj SS	*F*	*P*
Suppl/Unsuppl	1	2.72	1.04	0.31
Wl lesion	1	0.00	0.00	0.98
Wl damage	1	0.39	0.15	0.70
Left/right hind	1	0.20	0.07	0.79
Lat/Med claw	1	32.98	12.6	<0.01*
Zones 2/3	1	43.19	16.50	<0.01*

P* Significant difference

Analysis of variance (general linear model) for peak tensile strength (mega-pascals) of the white line for sample day 1

Variable	D F	Adj SS	F	P
Suppl/Unsuppl	1	1.81	0.78	0.38
Wl damage	1	7.04	3.04	0.08
Left/right hind	1	2.39	1.03	0.31
Lat/Med claw	1	7.56	3.26	0.07
Zones 2/3	1	115.64	49.95	<0.01*

P* Significant difference

Analysis of variance (general linear model) for peak tensile strength (mega-pascals) of the white line for sample day 2

Variable	D F	Adj SS	*F*	*P*
Suppl/Unsuppl	1	1.11	0.54	0.46
Wl damage	1	6.78	3.31	0.07
Left/right hind	1	1.46	0.71	0.40
Lat/Med claw	1	18.25	8.91	<0.01*
Zones 2/3	1	136.36	66.56	<0.01*

P* Significant difference

Analysis of variance (general linear model) for peak tensile strength (mega-pascals) of the white line for sample day 3

Variable	D F	Adj SS	*F*	*P*
Suppl/Unsuppl	1	0.00	0.00	1.00
Wl damage	1	7.58	3.26	0.07
Left/right hind	1	4.03	1.73	0.19
Lat/Med claw	1	20.47	8.80	<0.01*
Zones 2/3	1	82.36	35.43	<0.01*

P* Significant difference

Analysis of variance (general linear model) for peak tensile strength (mega-pascals) of the white line for sample day 4

Variable	D F	Adj SS	*F*	*P*
Suppl/Unsuppl	1	3.32	1.88	0.18
Wl damage	1	10.73	6.07	0.02*
Left/right hind	1	4.37	2.47	0.12
Lat/Med claw	1	3.69	2.09	0.15
Zones 2/3	1	38.11	21.57	<0.01*

P* Significant difference

Analysis of variance (general linear model) for peak tensile strength (mega-pascals) of the white line for sample day 5

Variable	D F	Adj SS	*F*	*P*
Suppl/Unsuppl	1	1.84	0.55	0.46
Wl damage	1	6.22	1.85	0.18
Left/right hind	1	24.82	7.40	<0.01*
Lat/Med claw	1	43.22	12.88	<0.01*
Zones 2/3	1	124.97	37.25	<0.01*

P* Significant difference

APPENDIX VI

Possible distinction between sole ulcers and heel ulcers as a cause of bovine lameness

R. W. BLOWEY, P. OSSENT, C. L. WATSON, V. HEDGES, L. E. GREEN, A. J. PACKINGTON

MOST authors consider that a sole ulcer is formed when excess pressure on the corium leads to bruising of the dermal papillae of the stratum germinativum with subsequent disruption of horn formation (Toussaint Raven 1985a, Blowey 1993, Collick 1997a, Ossent and Lischer 1998). The stratum germinativum is made up of the stratum basale and the stratum spinosum, these being the epidermal layers responsible for horn growth, where mitosis takes place. The epidermis itself cannot bruise because there are no blood vessels present.

The 'typical place' of the bruising of the dermal papillae is directly beneath the flexor tuberosity of the pedal bone (Toussaint Raven 1985a). Early theories suggested that exostoses on the third phalanx were an exacerbating factor (Rusterholz 1920). The nature of the suspension of the pedal bone within the hoof may also contribute. The laminar attachment of the pedal bone on the abaxial wall is much stronger and more extensive than its axial attachment; thus, the bone tends to rotate in an axial direction within the hoof when the foot is loaded, and this leads to further compression and damage to the corium (Toussaint Raven 1985a). A typical sole ulcer is situated in the axial region of zone 4 of the hoof (point A, Fig 1), classified according to the zones agreed at the Sixth International Lameness Symposium, Liverpool 1990, and described by Greenough and others (1997).

This short communication describes the incidence and possible aetiology of another lesion at a different and very specific area of the foot. The lesion was first proposed as a separate entity by one of the current authors (C. L. W.) during the standardisation of lesion description for a dairy cow lameness project (C. L. Watson, personal communication). Initially, the lesion was named 'necrotic heel track', which fitted the gross description of the lesion (Blowey and Hedges 1998). The term 'heel ulcer' was a later alternative based on its proposed aetiology.

The lesion is characteristically seen as a small deep purple or black mark approximately 2 to 3 mm in diameter in the central sole area at the junction of zones 4 and 6 (point B, Figs 1, 2). It therefore differs from the position of a 'typical' sole ulcer, which forms in the axial region of zone 4, and where the initial area of haemorrhage is much larger. If further slivers of the sole horn are removed from a heel ulcer, a track or fistula can often be followed passing obliquely towards the corium in a caudal direction. A small area of haemorrhage can be seen beneath the caudal edge of the lesion in Fig 2. In some cases the track fades and disappears before it reaches the corium, whereas in others it reaches the corium and appears as a small haemorrhagic area, as shown in the right claw of Fig 3. In lesions that cause pronounced lameness, the tract may expand into an area of separation of the horn from the underlying corium, and there is sometimes a discharge of haemorrhagic-suppurative exudate at the heel. It was because of these latter features that the term 'necrotic heel track' was given initially (C. L. Watson, personal communication).

In a population of 927 cows in five commercial dairy herds in Gloucestershire, all cases of lameness over an 18-month period were examined by a veterinarian. Details of the herds and the examinations have been given elsewhere (Hedges and others 1998). The 927 animals included all cows that were present at the start of the trial and all animals that entered the herd (calved in or purchased) during the period of the trial. A total of 766 cases of lameness were recorded and heel ulcers were identified in 63 cases, with considerable variation from farm to farm (Table 1).

Although 927 cows were involved in the trial, because some were calved in and others were culled, not all cows were present for the whole trial. To allow for this, the number of days each cow was present in the trial was calculated, and from this the total cow years (1104·6) involved in the trial. This figure was then used to calculate incidence based on cases per 100 cows per year.

The overall incidence of lameness was 69·3 cases per 100 cows per year. This figure is quite high, and considerably higher than figures of 54·6 cases per 100 cows per year quoted by Clarkson and others (1996), and 24·0 cases per 100 cows per year in a study of 90 DAISY recorded herds reported by Esslemont and Kossaibati (1996). The high incidence of lameness in herd 2 was associated with a very high frequency of both sole and heel ulcers, and particularly of 'typical' sole ulcers which failed to heal and produced large amounts of granulation tissue.

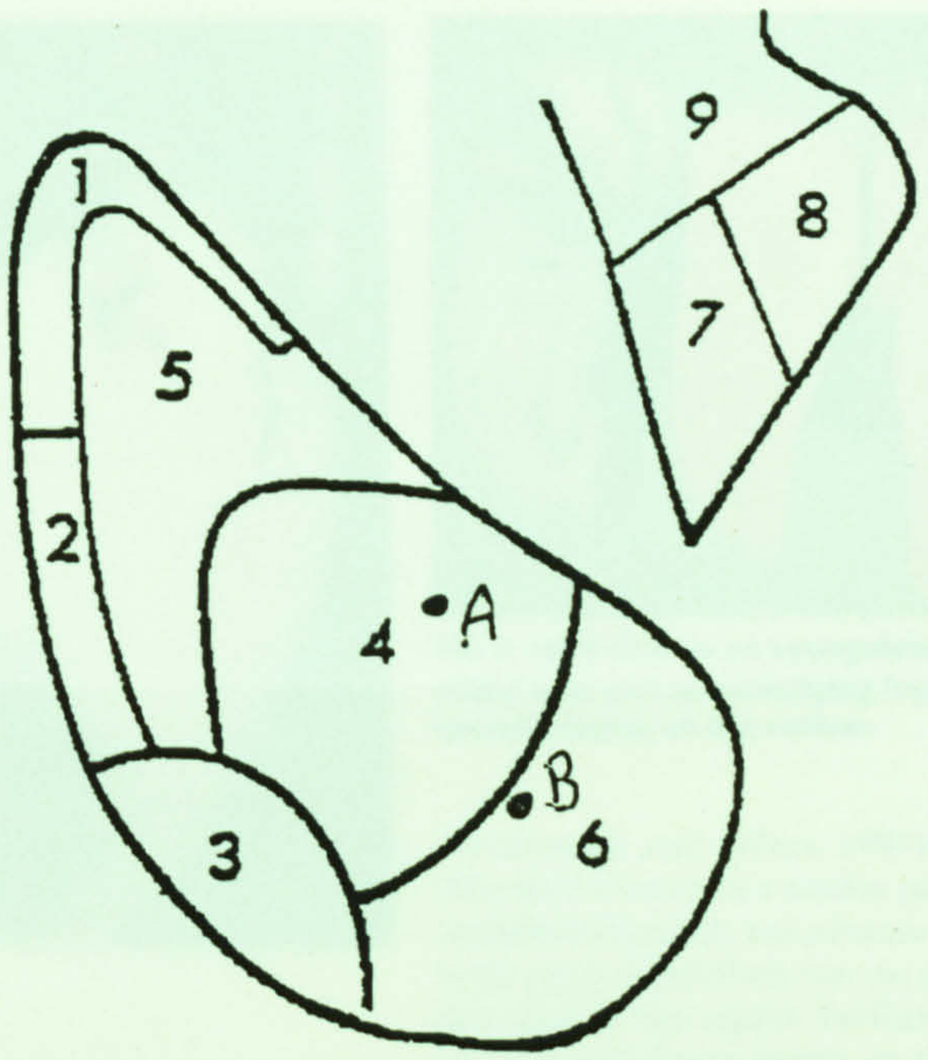


FIG 1: Diagram showing the zones of the sole and heel classified according to the Sixth International Lameness Symposium, Liverpool, 1990 (Greenough and others 1997). A Site of sole ulcer, B Site of heel ulcer

FIG 2: Early heel ulcer is seen as a small black lesion at the junction of zones 4 and 6 in the central sole area

Veterinary Record (2000) **147,** 110-112

R. W. Blowey, BSc, BVSc, FRCVS,
C. L. Watson, MA, VetMB, MRCVS,
V. Hedges, BSc, Wood Veterinary Group, 125 Bristol Road, Gloucester GL2 4NB
P. Ossent, DrMedVet, DipECVP, Institute for Veterinary Pathology, University of Zurich, Winterthurerstrasse 268, CH-8057, Zurich, Switzerland
L. E. Green, BVSc, MSc, PhD, MRCVS, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL
A. J. Packington, HND, Roche UK, Heanor Gate, Heanor, Derbyshire DE75 7SG

FIG 3: Removal of overlying sole to reveal a small area of exposed corium and under-run heel at the base of a heel ulcer

The distribution of lesions by claw is given in Table 2. The total number (571) of lameness cases in Table 2 is lower than Table 1 (766), because Table 2 records only claw-specific lesions, and does not include disorders such as interdigital necrobacillosis, digital dermatitis, mud fever and limb lesions. The high incidence of sole ulcers in the medial front claws and lateral hind claws is striking, and has been reported by previous authors (Russell and others 1982). Heel ulcers showed a similar predisposition towards the medial claw of the front feet, but there was no difference in the incidence in hindfeet. The numbers involved were too small for statistical analysis. The high incidence in the lateral claw of the right hindfoot cannot be explained. R. Pijl (personal communication) observed similar lesions during routine foot trimming of dairy cows in Germany, and considered that there was an increased incidence in the medial claws of front feet.

The heel ulcer lesion is not apparently described as a distinct entity in any standard texts on bovine lameness

FIG 4: Heel ulcer in an exungulated claw, showing penetration of the heel and an underlying focal area of haemorrhage and necrotic tissue on the corium

(Greenough and others 1997). Toussaint Raven (1985b) described a lesion in a similar position to a heel ulcer, but he considered that this was a fissure of the sole secondary to heel horn erosion and there was no similarity to the black fistula described in this report. Furthermore, the fissures described by Toussaint Raven began on the horn surface (since they were due to heel horn erosion), and only the most severe defects actually ulcerated. Another comparable lesion described by Toussaint Raven (1985c) was termed 'fracture of the horny sole'. In this condition, a wide flap of heel horn separates from the corium and runs longitudinally in a caudal direction across zones 4 and 6.

In his classification of lesions of the sole and horn bulb, Bergsten (1995) appears to refer to a condition similar to a heel ulcer as 'an under-run heel', but no specific catagory is defined. A heel abscess is undoubtedly one of the possible consequences of a heel ulcer, but this does not describe its aetiology. A discharging sinus at the heel is only rarely found as a sequel to heel ulcers, although many do discharge through the initial site of ulceration at the junction of zones 4 and 6. C. L. Watson (personal communication) initially considered that the lesion was a foreign body penetration of the sole and this was a view shared by P. R. Greenough (personal communication). Collick (1997b) also describes a very simi-

TABLE 1: Distribution of lameness, heel ulcers and sole ulcers in the five herds studied

Farm	Number of cows	Number of cases of lameness	Lameness cases/100 cows/year	Number of heel ulcers	Heel ulcers/100 cows/year	Number of sole ulcers	Sole ulcers/100 cows/year
1	174	64	31·1	4	1·9	10	4·9
2	184	269	110·9	47	19·4	91	37·5
3	238	161	59·3	2	0·7	19	7·0
4	220	228	91·3	6	2·4	44	17·6
5	111	44	32·5	4	3·0	12	8·9
Total	927	766		63		176	
Mean			69·3		5·7		15·9

TABLE 2: Distribution of heel ulcers and sole ulcers by claw (total population = 927 cows x 8 = 7416 claws)

	Front left		Front right		Hind left		Hind right		
Number of	Lateral	Medial	Medial	Lateral	Lateral	Medial	Medial	Lateral	Total
Claws	927	927	927	927	927	927	927	927	
Cases of lameness	17	42	43	11	168	49	51	190	571
Heel ulcers (%)	0	4 (0·43)	6 (0·65)	1 (0·11)	9 (0·97)	13 (1·40)	11 (1·19)	19 (2·05)	63
Sole ulcers (%)	4 (0·43)	13 (1·40)	12 (1·29)	2 (0·22)	63 (6·80)	8 (0·86)	6 (0·65)	69 (7·44)	177

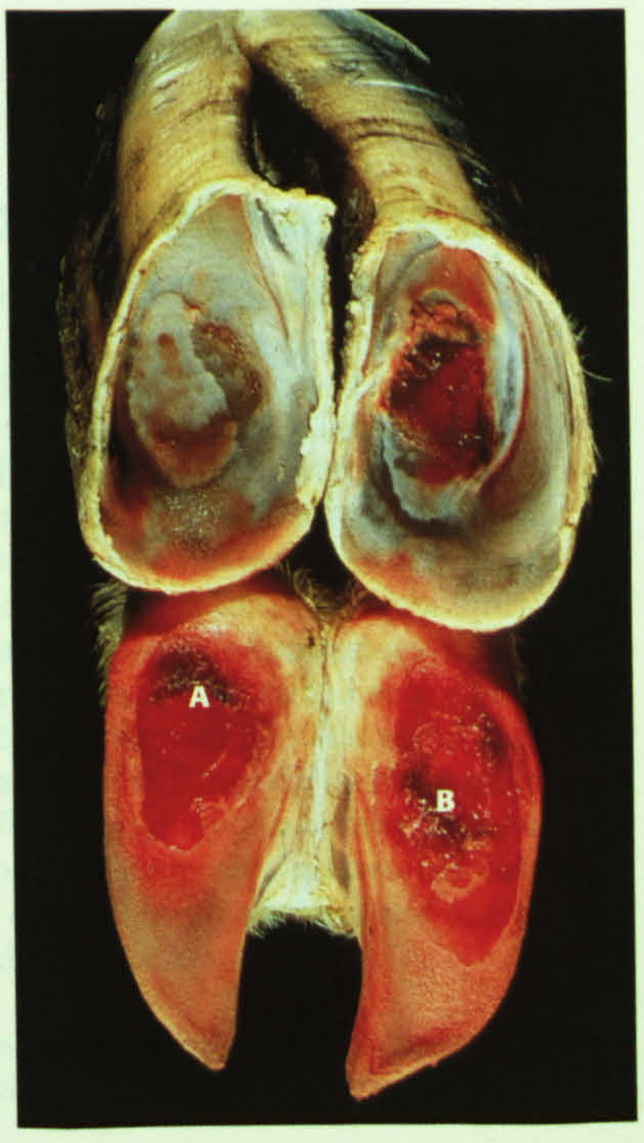


FIG 5: Claws of an exungulated foot showing the two distinct sites at which the heel ulcer (A) and the typical sole ulcer (B) develop from haemorrhagic necrotic foci in the corium, where it directly overlies the caudal egde of the third phalanx and the flexor tuberosity, respectively. The corresponding impressions are visible in the inner surfaces of the capsules. The sole and heel horn eventually perforate to form an ulcer if the damage persists for long enough

lar lesion in a report of a foreign body penetrating the sole. The case he refers to discharged at the heel in a manner identical to the severe forms of a heel ulcer. However, the foreign body penetration hypothesis does not account for the very specific site of the lesion, nor for the fact that it runs obliquely through the horn towards the heel.

In a photograph of an exungulated claw, Ossent and Lischer (1998) showed a heel ulcer very clearly as perforation of the sole horn, with underlying focal haemorrhage and necrosis (Fig 4) although these authors do not refer to the lesions specifically. The appearance of the dark red fistular defect in the horn strongly suggests that it developed in a similar way to the typical sole ulcer, namely, as a result of trauma to the underlying corium. The resulting haemorrhage and necrotic material is then incorporated into the horn and grows out to the surface diagonally, which is the normal direction of growth for the horn of the sole and heel. The ensuing defect in the heel presents a port of entry for secondary infection and the horn may become under-run or abscesses may develop within the bulb. Haematoma of the heel has been described recently (Blowey 1997), and although this lesion lies caudal to the site of a heel ulcer, there could be a similar pathogenesis, as suggested by the few cases where fine, layered haemorrhages grow out obliquely in a cranial direction from the heel horn. However, the most logical pathogenesis of heel ulcers is a pinching of the soft tissues of the corium by the caudal edge of the third phalanx. The typical sole ulcer develops by the same mechanism except that it is associated with trauma from the flexor tuberosity, and hence the site of a sole ulcer is slightly more axial and cranial than the site of a heel ulcer. These two distinct sites are clearly shown in Fig 5, where the early stages of both heel and sole ulcers may be seen in the corium of the same foot (Ossent 1998). A detailed examination of the original Rusterholz paper (Rusterholz 1920) indicates that this author was by no means specific about the site of a sole ulcer. Although all of his illustrations describe a sole ulcer in the 'typical place', the text refers to ulcers occurring at other sites. A translation from that text states: 'Or one encounters more in the middle, maybe also at any other part of the sole, a fistula-like opening with jagged edges, which, when explored with a probe, a canal may be followed in a caudal direction toward the medial edge of the hind end of the claw bone'.

There are many possible causes of trauma to the corium in this region of the hoof. These include excess standing, hoof overgrowth, uneven surfaces, rough handling of the cattle, and factors such as diet and parturition which increase the fragility of the corium (Ossent and others 1997, Friedli and Lischer 1998, Blowey 1999). Further studies are required to determine the precise aetiology of heel ulcers.

ACKNOWLEDGEMENTS

The authors would like to thank the participating farmers for their patience and perseverence, and Graham Stephens, Paul Cunliffe, and other members of the Wood Veterinary Group, for examining and recording lame cows. The work was financed by a grant from F. Hoffmann-La Roche.

References

BERGSTEN, C. (1995) Digital disorders in dairy cattle with special reference to laminitis and heel horn erosion. Dissertation to the Swedish University of Agricultural Sciences, Skara

BLOWEY, R. W. (1993) Cattle Lameness and Hoofcare. Ipswich, Farming Press p 11

BLOWEY, R. W. (1997) Haematoma of the heel, a potential cause of bovine lameness. *Veterinary Record* **140**, 507

BLOWEY, R. W. (1999) A Veterinary Book for Dairy Farmers. 3rd edn. Ipswich, Farming Press. p 293

BLOWEY, R. W. & HEDGES, V. J. (1998) Necrotic heel tract – is this a sole ulcer? ... 155

at a caudal site? Proceedings of the 10th International Symposium on Disorders of the Ruminant Digit, Lucerne, Switzerland, September 1998. pp 132-133

CLARKSON, M. J., DOWNHAM, D. Y., FAULL, W. B., HUGHES, J. W., MANSON, F. J., MERRIT, J. B., MURRAY, R. D., RUSSELL, W. B., SUTHERST, J. E. & WARD, W. R. (1996) Incidence and prevalence of lameness in dairy cattle. *Veterinary Record* **138**, 563-567

COLLICK, D. W. (1997a) Pododermatitis circumscripta (sole ulcer). In Lameness in Cattle. Eds P. R. Greenough, A. D. Weaver. Philadelphia, W. B. Saunders. p 101

COLLICK, D. W. (1997b) Traumatic injuries to the sole. In Lameness in Cattle. Eds P. R. Greenough, A. D. Weaver. Philadelphia, W. B. Saunders. p 114

ESSLEMONT, R. J. & KOSSAIBATI, M. A. (1996) The incidence of production disease and other health problems in a group of dairy herds in England. *Veterinary Record* **139,** 486-490

FRIEDLI, K. & LISCHER, C. (1998) Handbuch zur Pflege und Behandlung der Klauen beim Rind. Ed C. Lischer. Langenthal, Switzerland, Landwirtschaftliche Lehrmittelzentrale. p 105

GREENOUGH, P. R., WEAVER, A. D., BROOM, D. M., ESSLEMONT, R. J. & GALINDO, F. A. (1997) Basic concepts of bovine lameness. In Lameness in Cattle. Eds P. R. Greenough, A. D. Weaver. Philadelphia, W. B. Saunders. p 10

HEDGES, V. J., BLOWEY, R. W., PACKINGTON, A. J. & GREEN, L. E. (1998) A prospective study of causes of lameness in five herds of dairy cows: the first five months. Proceedings of the 10th International Symposium on Disorders of the Ruminant Digit, Lucerne, Swizerland, September 1988. pp 70-72

OSSENT, P. (1998) Handbuch zur Pfledge und Behandlung der Klauen beim Rind. Ed C. Lischer. Langenthal, Switzerland. Landwirtschaftliche Lehrmittelzentrale. p 139

OSSENT, P., GREENOUGH, P. R. & VERMUNT, J. J. (1997) Laminitis. In Lameness in Cattle. Eds P. R. Greenough, A. D. Weaver. Philadelphia, W. B. Saunders. p 277

OSSENT, P. & LISCHER, C. (1998) Bovine laminitis: the lesions and their pathogenesis. *In Practice* **20,** 415-427

RUSSELL, A. M., ROWLANDS, G. J., SHAW, S. R. & WEAVER, A. D. (1982) Survey of lameness in British dairy cattle. *Veterinary Record* **111** 155-160

RUSTERHOLZ, A. (1920) Das spezifisch-traumatische Klau ensohlengeschwur des Rindes. *Schweizer Archiv für Tierheilkunde* **62**, 505-525

TOUSSAINT RAVEN, E. (1985a) Cattle Footcare and Claw Trimming. Ipswich, Farming Press. p 25

TOUSSAINT RAVEN, E. (1985b) Cattle Footcare and Claw Trimming. Ipswich, Farming Press. p 95

TOUSSAINT RAVEN, E. (1985c) Cattle Footcare and Claw Trimming. Ipswich, Farming Press. p 63

ABSTRACT

Magnetic resonance imaging of orbital disease in dogs and cats

TWENTY-TWO dogs and three cats with signs of orbital disease were investigated by magnetic resonance imaging (MRI) and ultrasonography, and skull radiographs were taken of 17 of the dogs and the three cats. The MRI scans produced detailed images of the orbital tissues and indicated the extent of the pathology more accurately than the other techniques; correct diagnoses, including neoplasia, inflammatory disease and penetration by foreign bodies, were obtained on the basis of the MRI scan alone in 22 cases. Radiography was useful only when neoplastic disease extended well into the nasal chamber and paranasal sinuses. Ultrasonography gave false positive and false negative diagnoses for neoplastic masses, but correctly diagnosed the two cases of foreign bodies and one of the three cases of retrobulbar abscesses.

DENNIS, R. (2000) Use of magnetic resonance imaging for the investigation of orbital disease in small animals. *Journal of Small Animal Practice* **41,** 145-

UNIVERSITY OF BRISTOL LIBRARY MEDICAL